QTL mapping in *Hevea brasiliensis* for analysing the genetic determinism of growth, latex production, and the macromolecular structure of natural rubber

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Abstract

This research developed the first QTL-mapping approach on the domesticated population of the rubber tree (*Hevea brasiliensis*), based on a F1 family issued from two widely cultivated rubber varieties (RRIM600 x PB217). Genetic mapping (Prapan et al. 2004) was carried out by use of 427 PCR-based molecular genetic markers: 243 SSR (microsatellites) and 184 AFLP. Phenotyping was carried out over 196 progenies, with 16 budded copies per genotype, in East-Thailand on a site regularly affected by water stress. The measurements covered a period of 5 years of initial growth, followed by three tapping periods during the rainy seasons of three successive years, with an increasing tapping frequency and the application of ethephon stimulations. The studied traits were : growth in height and growth in girth before and during tapping, defoliation earliness, scoring of a die-back phenomenon in response to a severe drought, latex production, latex diagnostic (Drc, Suc, Pi, Rsh), plugging index (W1, W2, PI), bark thickness, leaves dimensions, and the molar mass distributions of the rubber chains which were measured by Steric Exclusion Chromatography.

Among 48 QTLs, two major QTLs were detected repeatedly. Among 12 QTLs, the QTL g3-60 was associated with growth in girth of the trunk, with the highest effect during the most favourable periods, and it explained up to 31 % of the phenotypic variance. Among 11 QTLs, the QTL g16-6 was associated with latex production and the related traits such as inorganic phosphorus, dry rubber content, and sucrose content of the latex, and it explained up to 66 % of the phenotypic variance. Intensification of the tapping system generated a strong general reduction in the genetic variability of production traits, and a related reduction in the effect of the QTL g16-6, whereas the influence of trunk girth and of the QTL g3-60 on production was increased, thus indicating the beginning of a change in the genetic determinism of the production due to intensification. Only one other QTL (g16-46) was detected specifically for sucrose content traits. The QTL g1-80 was detected repeatedly for thiol content traits. The cumulated effect of five QTLs explained 55 % of the variance of the percentage of short rubber chains which is indicative of the monomodal or bimodal distributions of the molar masses of native rubber.

These results are important for the understanding of the genetic determinism of traits of interest, the application of Markers-Assisted Selection (MAS), and candidate-gene mapping. A new system of early selection is proposed, combining QTL genotyping and field phenotyping for increasing genetic progress in selection.

Keywords *Hevea brasiliensis*, natural rubber, rubber breeding, Wickham population, heritabilities, correlations, genetic mapping, QTL-mapping, growth, latex production, plugging index, latex diagnostic, molar mass distributions, Steric Exclusion Chromatography (SEC), Markers-Assisted Selection (MAS).

Résumé en français

Cette recherche concerne la première cartographie de QTLs sur la population domestiquée de l'hévéa (*Hevea brasiliensis*), à partir d'une famille F1 issue de deux variétés très cultivées (RRIM600 x PB217). La cartographie génétique (Prapan et al. 2004) a été réalisée avec 427 marqueurs génétiques issus de la technologie de PCR ciblée : 243 SSR (microsatellites) et 184 AFLP. Le phénotypage a été réalisé sur 196 descendants comportant 16 copies greffées par génotype, dans l'est de la Thaïlande sur un site régulièrement affecté par des stresses hydriques. Les mesures ont porté sur la croissance initiale (5 ans), puis sur trois périodes de saignée pendant les trois années suivantes, avec une fréquence de saignée croissante et l'application de stimulations à l'ethephon. Les principaux caractères mesurés étaient : la croissance en hauteur et en circonférence du tronc avant et pendant la saignée, la précocité de défoliation, les notations d'un phénomène de die-back en période de sécheresse, l'épaisseur d'écorce, des caractères de dimension foliaire, la production de latex, les caractères du diagnostic latex (Drc, Suc, Pi, Rsh) et du plugging index (W1, W2, PI), et les distributions de masses molaires du caoutchouc natif mesurées par chromatographie d'exclusion stérique.

Parmi 48 QTLs, deux QTLs majeurs ont été détectés de façon répétée. Parmi 12 QTLs, le QTL g3-60 était associé à la croissance en circonférence du tronc, avec un effet maximum pendant les périodes les plus favorables, expliquant jusqu'à 31 % de la variance phénotypique. Parmi 11 QTLs, le QTL g16-6 était associé à la production et aux caractères corrélés tels que le taux de caoutchouc sec et la durée d'écoulement du latex après la saignée, le taux de phosphore inorganique, et le taux de saccharose dans le latex, et son effet expliquait jusqu'à 66 % de la variance phénotypique. L'intensification du système de saignée a produit une forte réduction générale de la variabilité génétique des caractères de production et une réduction conjointe de l'effet du QTL g16-6, tandis que l'influence de la circonférence de tronc et du QTL g3-60 sur la production était accrue, indiquant ainsi le début d'un changement du déterminisme génétique de la production du fait de l'intensification. Un seul autre QTL (g16-46) a été détecté spécifiquement pour la teneur en saccharose du latex. Le QTL g1-80 a été détecté de façon répétée pour la teneur en groupements thiols du latex. L'effet additif cumulé de cinq QTLs expliquait 55 % de la variance phénotypique du pourcentage de chaînes courtes de caoutchouc, pourcentage indicatif du caractère monomodal ou bimodal des distributions de masses molaires du caoutchouc natif.

Ces résultats sont importants pour la compréhension du déterminisme génétique des caractères d'intérêt, l'application de la Sélection Assistée par Marqueurs (SAM) et la cartographie de gènes candidats. Une nouvelle sélection précoce est proposée, combinant génotypage aux QTLs et phénotypage en champ afin d'augmenter le progrès génétique.

Mots-clés *Hevea brasiliensis*, caoutchouc naturel, amélioration génétique de l'hévéa, population Wickham, héritabilités, corrélations, cartographie génétique, cartographie de QTLs, croissance, production de latex, plugging index, diagnostic latex, distributions des masses molaires, Chromatographie d'Exclusion Stérique (CES), Sélection Assistée par Marqueurs (SAM).

The results from this thesis will be published as following :

Genetic determinism of natural rubber production analysed by QTL-mapping in the domesticated population of *Hevea brasiliensis*.

R. Rattanawong, K. Prapan, N. Lekawipat, K. Teerawatanasuk, P. Kasemsap, T. Toojinda, M. Seguin, A. Clément-Demange.

Pre-tapping growth of the rubber tree analysed by QTL mapping in the domesticated population of *Hevea brasiliensis*.

R. Rattanawong, K. Prapan, N. Lekawipat, K. Teerawatanasuk, P. Kasemsap, T. Toojinda, M. Seguin, A. Clément-Demange.

Native rubber macromolecular structure analysed by Steric Exclusion Chromatography and QTL-mapping in the domesticated population of *Hevea brasiliensis*.

R. Rattanawong, E. Delpuech, C. Char, K. Teerawatanasuk, P. Kasemsap, T. Toojinda, M. Seguin, F. Bonfils, A. Clément-Demange.

Genetic mapping was a pre-requisite to this research. It will be published by K. Prapan et al. :

PCR-based genetic mapping of a F1 population derived from two rubber cultivars of the domesticated population of *Hevea brasiliensis*.

K. Prapan, N. Lekawipat, C. Weber, R. Rattanawong, K. Teerawatanasuk, T. Toojinda, P. Kasemsap, A. Clément-Demange, M. Seguin.

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INTRODUCTION

This genetics research developed the first QTL-mapping approach on the domesticated Wickham population of the rubber tree (*Hevea brasiliensis*), for application to rubber breeding by Markers-Assisted Selection (MAS). It followed and took advantage of the pioneering work which was developed in interspecific progenies for analysing the genetic variability of rubber response to the South-American Leaf Blight (Lespinasse et al. 2000a, 2000b, Le Guen et al. 2003, 2007). It was based on a F1 family issued from two widely cultivated rubber clones (RRIM600 x PB217). Genetic mapping (Prapan et al. 2004) was carried out by use of 427 PCR-based molecular genetic markers (243 SSR and 184 AFLP). Although a pre-requisite, this genetic mapping, made by another researcher, is not part of this PhD research devoted to phenotyping and QTL mapping. Phenotyping was carried out on 196 progenies in East-Thailand over the period from 2002 to 2009. The target traits were related with ecophysiology (growth), latex cell physiology (rubber production), and rubber quality (monomodal or bimodal distributions of the molar masses of the rubber chains). The choice of a F1 family was adapted to the rubber tree, a tree crop and an outbred species, with vegetatively propagated varieties in the form of heterozygous clones budded onto seedling rootstocks. This “Genmap” project was one of the French-Thai Hrpp projects (Hevea Research Programme in Partnership), associating Rrit-Doa, Kasetsart University, and Cirad. Thailand is the largest rubber producing country, with a cultivated area of more than 2.4 million hectares, and a production of 3.08 million tons of rubber in 2008, as compared to a total world production of 9.94 MT. Therefore, improving the efficiency of rubber breeding and delivering performant and adapted clones to the planters is an important economical objective for Thailand.

The objectives of this research were as following :

- One general objective was to develop the applications of molecular genetic markers to rubber breeding. Some of these applications have recently been

explored in Thailand such as genetic diversity analysis and clonal identification (Lekawipat et al. 2003a, 2003b).

- The initial objective was to analyse the genetic determinism of the traits associated with the biochemical factors of the « latex diagnostic ». In particular, this included sucrose rate in the latex, which is considered as a determinant of the potential of production of the clones in the long term. This objective determined the choice of the two contrasted parents, RRIM600 (quick starter), and PB217 (slow starter).

- This objective was extended to investigations on the genetic determinism of rubber agricultural traits by QTL detection. As far as a genetic map was built for the F1 family, any other measurable trait could be submitted to QTL detection ; therefore investigations were enlarged to growth before tapping and to natural rubber quality, in the limits of our measurement capacities.

- This research was targetted towards the improvement of the efficiency of the first step of the clonal selection, by application of Markers-Assisted Selection (MAS). This could be achieved through the identification of molecular selection criteria, independent from the environment and able to increase the accuracy of estimation of the genetic values of the candidates to selection.

- This research should also contribute to global research in rubber genetics by preparing the possibility of candidate gene-mapping and of the selection on the allelic forms of key-genes for traits of interest.

- The last objective was to select from 5 to 20 new clones among the 196 tested genotypes, just as in a classical small scale clonal trial. For rubber as for any tree crop, due to the long duration of breeding and selection, it is always highly wishable to design experiments for both methodological and operational objectives.

1. LITERATURE REVIEW

1.1. The rubber tree

The genus *Hevea* belongs to the Euphorbiaceae family, and includes 10 species. It is indigenous to the tropical humid forests of the Amazon Basin. Natural rubber was first collected from wild trees by « seringueiros ». Since 1876, after the transfer of plant material to Asia, the rubber tree was domesticated and cultivated. The taxonomy of this genus was described by Webster (1975) and Schultes (1990). *Hevea* (2n = 36, x = 9) behaves as a diploid plant. It is monoecious, outcrossing, and highly heterozygotic. *Hevea* can be easily recognised by its trifoliated leaves and its fruits most usually made of three seeds.

The species *Hevea brasiliensis* is quite exclusively cultivated. It grows best at temperatures of 20-28°C with a well-distributed annual rainfall of 1800 mm. It grows satisfactorily up to 600 metres above sea level, and performs on most soils provided drainage is adequate. Rubber is cultivated until extreme latitudes of 20 ° on either side of the equator. Natural rubber is a polymer [$(C5H8)_n$], and its main property, elasticity, is improved and maintained by vulcanisation. Rubber cropping is also valued for wood that becomes available after felling. Rubberwood may represent about 15% of the total income of the farmers (Clement-Demange et al. 2003). Although initial cultivation took place on large plantations (estates), smallholdings currently represent 80 % of the total rubber cropping area. Rubber is produced mainly in Southeast Asia (92%), and also in Africa (6%) and Latin America (2%). The main producing countries are Thailand, Indonesia, Malaysia, India, China, Vietnam, Côte d'Ivoire, Liberia, and Sri-Lanka (IRSG 2009).

Varieties are budded clones, with leaf axillary buds grafted onto seedling rootstocks. Every clone is issued from one initial seed which is obtained by natural pollination or hand pollination in breeding programs. Budwood gardens are developed for maintaining the collections of clones and for producing the buds used for budding the rootstocks in nurseries. Rootstocks are grown from heterogeneous non-selected seeds collected in commercial plantations. As a result, each budded tree is made of two components, the clonal aerial part,

resulting from intensive selection, and the unselected rootstock. Cloning the root system is very sensitive to the juvenility of plant tissues, and research is still thriving for developing clonal rootstocks.

After six years, the trees normally reach a trunk girth of 50 cm and become ready for “opening” (tapping becomes economically feasible). Latex production is progressively increased during the first five years of tapping, and then follows fluctuations depending on the tapping system and the metabolic type of the clone. Whereas latex production per tree is only indicative of the tree potential, the production per land unit is largely related with the stability of the tapping stand along time (resistance to diseases, Tapping Panel Dryness, wind damage, etc.).

Latex vessels accessible for tapping are located in the bark of the trunk. Latex is not a sap but the cytoplasm of the anastomosed latex cells. It is a colloidal suspension of rubber particles which make 90 % of the total solid content of the latex. Latex is collected by periodic tapping of the bark every two, three, four or five days. Stimulation by ethephon generates ethylene that emphasizes latex flow after tapping, and regeneration between two tappings. Tapping systems combine varied elements such as the length of tapping-cut, the tapping frequency, the stimulation concentration and frequency, and the multi-annual management of the tapping-panel of the trunk. Tapping generates a competition for carbohydrate assimilates between latex regeneration and the growth of the tree, with a resulting strong reduction in trunk girth increment during tapping.

Rubber was first planted in Thailand in 1899 by Phraya Ratsadanupradit, Trang’s provincial governor. He brought the plant from Malaya and initially planted it in Kan Tang district of Trang province. Rubber plantations were promoted only recently in the eastern region (since 1978). In 1961, the government set up the Office of Rubber Replanting Aid Fund (ORRAF) to promote clonal rubber planting in Thailand (clone RRIM600). In 2009, rubber was cultivated quite exclusively on smallholdings, with 67 % located in the peninsular South, 12 % in the central part of the country, 17 % in the Northeast, and 4 % in the North (Office of Agricultural Economics, Thailand).

1.2. Rubber physiology

1.2.1. Growth and development

Rubber tree has been used as the model plant for designing the Rauh's architectural model (Hallé et Martin 1968), mainly characterized by rythmic growth and growth units. Rubber growth was studied from different point of views:

- Modelling of growth before tapping (Shorrocks 1965; Templeton 1968)
- Tree and stand growth modelling (Shorrocks 1965; Saint-André et al. 2005)
- Architectural modelling (Hallé and Martin 1968; Costes and de Reffye 1990)
- Ecophysiological funtionning (Sethuraj 1992; Sangsing 2004)

1.2.2. Rubber production

Anatomy of the bark and of the laticifer system has been described first by Bobilioff (1923) and by Gomez (1982). This laticifer system is developed in the soft bark of the trunk from which latex can be extracted by tapping. Upon tapping, nuclei and mitochondria remain adherent to the plasmalemma, thereby making possible the latex regeneration after tapping.

Rubber production results from the cycle "Latex flow – Coagulation – Regeneration" which is maintained by periodic tapping :

- Initial latex flow is caused by turgor pressure in the laticifers, which is as high as 10–14 atmospheres before sunrise. Water plays a key role in the duration of latex flow.

- Coagulation on the tapping cut stops latex flow. Lutoids contain coagulating factors. The rupture of their membranes, facilitated by the high osmotic gradient near the cut, leads to laticifer plugging (Southorn and Edwin 1968).

- Regeneration of the latex between two tappings is related with the cellular metabolism of the laticifer system and with the ecophysiological functioning of the tree. The full regeneration of the latex after one tapping was estimated to be around 72 hr (Serres et al. 1994).

1.2.3. Ethylene and latex production

Ethylene (C_2H_4), a “stress hormone”, induces many effects in plants such as fruit ripening, leaf abscission, stress and pathogen responses. Its role in rubber production is particularly important. Ethylene biosynthesis is triggered by various developmental processes, or by environmental stresses such as wounding, flooding, chilling, disease, high temperature, or drought stress. In rubber, ethylene biosynthesis is triggered by tapping stress and by exogenous ethylene. Stimulation of the bark, based on the provision of ethephon, the 2-chloroethyl-phosphonic acid which is an ethylene-releasing compound (Abraham et al. 1968), emphasizes ethylene biosynthesis (autocatalytic reaction). Stimulation increases rubber production by affecting latex flow, coagulation and latex regeneration, and it has become a powerful tool for monitoring rubber production systems.

Ethylene triggers complex mechanisms of signalling and of gene expression in the latex cells. This results in the increase in latex pH, metabolic activity, adenyl nucleotide content (energy production), invertase activity (sucrose consumption in latex cells), thus increasing latex regeneration. This higher pH also increases the lutoid membrane stability, resulting in a delay of coagulation during 10 tappings after treatment (Ribaillier 1972). Ethylene increases the sink effect of the laticifer with respect to sucrose loading and water loading from apoplast to laticiferous cells, thus increasing latex regeneration and the duration of latex flow. The effect of stimulation can be perceptible as soon as 12 hours after treatment, it is maximum 48-72 hours after stimulation, and can be still effective after 3 or 4 weeks (d’Auzac et al. 1997). The intensity and duration of the laticifer response to ethylene is a clonal characteristic. Excessive stimulation generates long term negative effects on latex production by oxidative stress and tapping cut dryness, with marked clonal differences.

1.2.4. Latex diagnostic and the metabolic typology of the clones

A review (d’Auzac et al. 1997) presents the concept of the physiological typology of the clones, based on the metabolic activity of the laticiferous cells, the provision of these cells with sucrose, and their protection from oxidative stress. The “latex diagnostic” (LD) was developed, made of the measurements of the Dry Rubber

Content (Drc), the Sucrose Content (Suc), the Inorganic Phosphorus Content (Pi), and the Thiols group Content (Rsh) in the latex (Jacob et al. 1987). Correlations were found between these physiological traits and latex yield, especially a positive correlation between Pi and latex production, and the clonal nature of these traits was established (Eschbach et al. 1984). LD was first developed for optimizing the tapping systems and monitoring commercial plots under tapping. A clonal metabolic typology of the clones was developed and applied to the adjustment of stimulation intensity depending on the clone (Jacob et al. 1989; Gohet et al. 2003).

- Dry Rubber Content (Drc), dependent on rubber biosynthesis intensity, strongly influences the length of latex flow: Lower the level of Drc and longer the latex flow. Thus, paradoxically, a low Drc content results in a higher dry rubber production due to the so-called “dilution effect”.

- Sucrose in the tree is issued from photosynthesis, and from the mobilization of starch reserves of the wood of the trunk. In the latex cells, sucrose is both the raw material for rubber biosynthesis and energy production (ATP) by respiration. The latex sucrose content depends on the balance between its influx from the apoplast and its use in the laticifers (d’Auzac et al. 1997). Therefore a high sucrose content in the latex can indicate either (i) a high influx of sucrose, or (ii) a poor utilization for rubber production. When sucrose availability is the limiting factor of rubber production, there is a positive correlation between Suc and latex yield. When sucrose utilization is low, sucrose accumulates and the correlation is negative. Excessive exploitation and the often subsequent onset of Tapping Panel Dryness may raise sucrose levels to above those found in normal trees.

- Inorganic phosphorus (Pi) in the latex cells comes mainly from the hydrolysis of the pyrophosphates which are involved in the biosynthetic lengthening of the polyisoprene chains. As a consequence, it is indicative of energy metabolism and isoprenic anabolism. There is always a high positive correlation between Pi and latex production, within and between clones. Pi content is increased by ethrel stimulation and decreased during wintering.

- Thiol (Rsh) in the latex is assumed to reflect the level of tolerance of latex cells to oxidative stress. Thiols (e.g. glutathione) present in the latex can neutralize toxic oxygen and activate some detoxicating enzymes. However experience indicated a more complex situation. Therefore this trait is considered as less important than the three others.

The LD analyses are based on colorimetric reactions (Ashwell 1957 for sucrose content, Taussky and Shorr 1953 for inorganic phosphorus content, and Boyne and Ellman 1972 for thiol content). The levels of Suc and Pi, measured in the absence of ethephon stimulation, have made possible the early characterization of the clones (Gohet 1996). Quick-starters (high Pi and low Suc) such as RRIM600 exhibit a naturally high metabolic activity, resulting in a high latex production during the first years of tapping and a low response to ethephon stimulation. Generally these clones have a limited latex production potential in the long run. By contrast, slow-starters (low Pi and high Suc) such as PB217 can be activated by ethephon stimulation and sometimes show a high production potential in the long run.

1.2.5. Plugging index

The plugging index (PI) accounts for the rate of laticifer closing by latex plugs. It involves initial turgor pressure in the laticifers and the total duration of latex flow. Latex flow is rapid immediately after tapping but quickly slows down and eventually ceases at the time of coagulation on the tapping-cut. Clones with a low PI tend to have a long duration of latex flow. Ethylene stimulation, that prolongs the duration of latex flow, reduces PI significantly. PI, with large differences between clones, can be used as a selection trait (Milford et al. 1969).

The standard method of PI determination involves the measurement of latex volume over the initial period of 5 minutes just after tapping, and the measurement of the total volume from tapping to coagulation. PI is calculated as following :

- Initial flow rate = (Latex vol. over first 5 mins of tapping ÷ 5) ml/min.
- PI = (Initial flow rate) ÷ (Total latex vol.) x 100.

1.2.6. Tapping Panel Dryness (TPD)

TPD is a specific issue of the rubber tree, resulting in the cessation of latex flow of some trees. It starts from nil at opening, and progressively affects the rubber stand to a varying rapidity. Attention was first drawn on seedlings (Rutgers and Dammerman 1914, Sanderson and Sutcliffe 1921), to the browning of the inner part of the bark, its necrosis and progressive destructuration, and this phenomenon was called the “Brown Bast disease” (de Faÿ and Jacob 1989, Eschbach et al. 1989). But the development of “tapping cut dryness” without any sign of browning was also observed. In severe cases the tapping cut may even become completely dry. In IRRDB meetings, the generic term TPD was used for the two syndromes (tapping cut dryness and brown bast). It was found that tapping cut dryness was reversible, depending on tapping intensity and seasonal variations, whereas brown bast was quite irreversible and definitely led to the loss of tapped trees (Jacob et al. 1994). Although exceptional cases were reported, brown bast do not develop on untapped trees, and it seems highly probable that tapping stress (wounding) is the major causal factor affecting the susceptible clones.

1.2.7. Water stress

With global heating and rubber development in suboptimal areas, tolerance to water stress has become a major concern. It mainly concerns survival of the trees, especially at young age, and growth fastness until opening. Apart from the importance of water for evapotranspiration and photosynthesis, water is also qualitatively very important for latex flow (Buttery and Boatman, 1976). Varied ecophysiological studies were carried out on rubber response to water stress, by Chandrashekar et al. (1994, 1996, 1997), Ceulemans et al. (1983), Devakumar et al. (1988, 1998a, 1998b), Gururaja Rao et al. (1986, 1988, 1990), Pakianathan et al. (1989), Rao (1990), Samarappuli (1998), Thomas et al. (1996), Vijayakumar et al. (1998). But there is still no clear criteria for early selection of rubber tolerance to water stress. Nowadays, it would still be quite impossible to make clonal recommendations for water stress-affected areas with reliable arguments.

The clone RRIM600 was assumed to be tolerant to water stress (Jacob et al. 1999). In Thailand, it was reported as growing moderately in the dry seasons, when most other clones had stopped their growth (Wichitchonchai and Manmuen

1992 ; Manmuen et al. 1993). Sangsing (2004a) studied rubber carbon acquisition and plant water status in response to water stress, for a set of clones on young plants in pots. Xylem conduction and cavitation in rubber were first studied by Ranasinghe and Milburn (1995). Rubber xylem embolism was studied in relation with xylem pressure and stomatal conductance for the two clones RRIM600 and RRIT251 (Sangsing 2004b). As a species of the humid tropics, rubber was found highly susceptible to embolism as compared with temperate species.

1.2.8. Molar mass distribution of the rubber chains

Due to the biological origin of natural rubber, its structure is much more complex than that of synthetic polymers. The causes of variability of natural rubber properties come, among others, from the clonal influence. Thereby, rubber quality should be taken into account for clonal recommendations. Due to their technological properties, some clones, such as PB217, cannot be used for producing centrifuged latex. For some other clones planted by smallholders in remote areas, the storing of coagulated rubber at farm level during three of four weeks (maturation) leads to a fast degradation of rubber quality, especially a higher sensitivity of rubber to oxidation (Intapun 2009).

Bonfils and Vaysse divided the structure of natural rubber into three main levels :

- Microstructure concerns the molecular level and chemical structure
- Mesostructure concerns the « macromolecular » structure of rubber, mainly characterized by its molar mass distribution (MMD) and its gel content. These characteristics have influence on viscosity and hardness.
- Macrostructure takes into account the characteristics observed in the whole product such as rheology (viscoelasticity and plasticity), breakdown behaviour, and vulcanization.

Gel is the rubber part staying insoluble in organic solvents. Macrogel can be eliminated by centrifugation, whereas microgel is eliminated by filtration (porosity $\leq$ 1 µm) (Bonfils et. al, 2005). The gel fraction results from branching phenomenons. The macrogel content decreases on every tapping and reaches a value of about 5% on regular tapping (Sekhar 1962). During storage of natural rubber, gel content

increases and may reach 50% or even higher after a long storage (Subramaniam, 1987). Gel content can also be increased by other factors such as clonal characteristics and rubber process (Dogadkin and Kuleznev 1960; Ngolemasango *et al* 2003; referenced by Liengprayoon 2008).

Natural rubber has a very high molar mass, with chains varying from 0.01 x 10^6 to 10 x 10^6 grammes/mole. In fact, there is variation in molar mass within each rubber sample. Even if the number of long chains is always less important than the number of short chains, the relative weight of the long chains play a prominent role in the rheological (or mechanical properties of rubber. The distribution of molar mass is generally shown in “weight” (weight plotted against molar mass).

Observation of the molar mass distribution of the rubber chains is generally carried out on native rubber, which is fresh latex just extracted from the trees prior to any modification of the charactreistics of the rubber polymer, or in CV rubber, with viscosity stabilized by hydroxylamine sulphate. Native rubber shows monomodal or bimodal distributions of “molar mass in weight”, depending on the clones. A monomodal distribution indicates a relatively higher number of long chains. This variation is no more visible in rubber issued from natural coagulation and maturation of coagula (TSR10 or TSR20): in this case, all the clones exhibit a monomodal distribution. But clones with a bimodal distribution of native rubber (RRIM600, GT1, or PR107) tend to produce a harder commercial rubber than that of clones with a monomodal distribution (PB217). Normally, in commercial production, lattices from several clones are mixed and bulked before drying in order to level out the clonal differences and reduce their effects.

Before the years 1960’s, molar masses of polymers were determined by osmometry (M_n, in “number of chains”), by light diffusion (M_w, in “weight”), or by centrifugation (M_z, in “volume”). For rubber solutions, it was also possible to measure an average viscosimetric molar mass (M_v) which was intermediate between M_n and M_w (Bonfils, personal communication). Nowadays molar mass distribution (MMD) in polymer systems is currently determined by Size Exclusion Chromatography (SEC), formerly called Gel Permeation Chromatography (GPC). This method of liquid chromatography separates macromolecules according to

their molecular sizes (Malawer et al. 1995). A liquid mobile phase is passed through a column at a fixed flow rate. The particles in the column are porous with controlled pore size. The smaller macromolecules are able to penetrate these pores and are delayed due to a longer way to go through, but the larger ones remain in the intersticial space and flow more rapidly down the length of the column. As a result, long chains are eluted first, followed by small chains. The raw chromatogram obtained as output from the concentration detector is divided into a number of time slices of equal width. An average molecular weight is assigned to each time slice based upon the calibration curve. For each time slice, the concentration detector provides a signal in millivolt, equivalent to a concentration in mg per ml of elutant. As a result, the area of each time slice is equivalent to a weight.

MMD_0, as measured on films prepared from fresh latex just extracted from the trees (native rubber), is an important clonal parameter for the prediction of some properties of commercial natural rubber. Subramaniam (1972) first studied MMD_0 by SEC analysis. He showed large differences between clones for molar mass distributions in weight (figure 1). Whereas the monomodal distribution described as type 3 has a high peak in the high molar mass region, and a shoulder in the low molar mass region, the bimodal distribution may be of two types: one of type1 where the lower and higher molar mass peaks have nearly the same height, and the other of type 2 (intermediate type) where the height of the higher molar mass peak is larger than that of the lower molar mass peak.

Figure 1 : a) Types of molar mass distribution curves of natural rubber ; b) Molar mass distribution of RRIM703 (type 1) ; c) Molar mass distribution of PB5/51 (Type 3). From Subramaniam 1976.

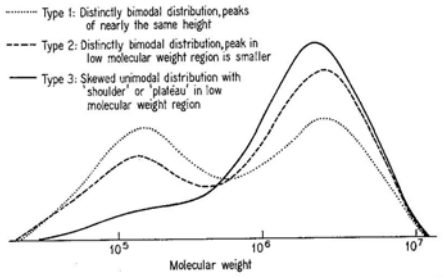


Figure 1. Types of molecular weight distribution curves of natural rubber.

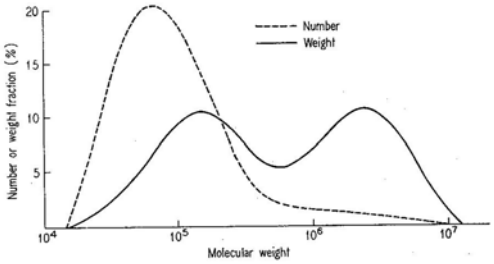


Figure 2. Molecular weight distribution for RRIM 703 (Type 1).

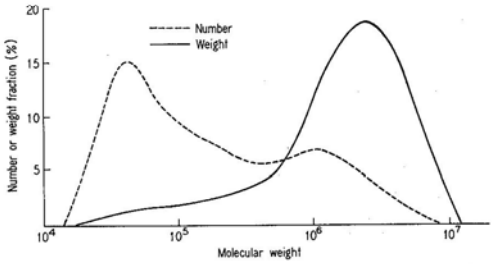


Figure 3. Molecular weight distribution for PB 5/51 (Type 3).

Clonal rubbers with low or average molar mass showed distinct bimodal distributions (Subramaniam 1976). The clonal rubbers with high average molar mass usually showed a monomodal distribution with a “shoulder” in the low molar mass region. The polydispersity index ($I_p = M_w / M_n$) varied from 2.8 to 10. It is an indicator of the dispersion of the distribution. The number-average molar mass (M_n) varied from 0.195 to 0.521 x 10^6 g/mole, and weight-average molar mass (M_w) varied from 1.66 to 2.28 x 10^6 g/mole. The clones giving rubbers with high molar masses, viscosities, and plasticities were PB28/59, PB5/51, PB5/63, TJIR1, and RRIC36. The clones showing the lowest values of these parameters were derived from RRIM703, RRIM605, and RRIM501.

Bonfils et al. (1995) studied the conditions of SEC analysis as applied to native rubber of the clones AVROS2037 and RRIM600. They used cyclohexane solvent

in the elution phase instead of tetrahydrofuran, and they were confronted with gel rates as high as 50 %. This high gel rate obviously reduces the amount of rubber solution available for MMD determination. This problem was solved by adding hydroxylamine neutral sulphate (HAS) to the latex prior to film preparation, or by washing the films in deionized water before dissolving them. Bonfils et al. (2005) presented the results of latex films prepared from 1 to 18 months after tapping initiation for the five clones GT1, VM515, PB312, RRIC121, and PB330. The initial macrogel was very high (70-86 % depending on the clone) but it fell progressively along the 18-month period. During the same period, the initially low microgel rate increased and stabilized at around 55 % of the total rubber. During this period, M_w (weight-average molar mass) increased and stabilized after 7 months of tapping.

In young trees, an original situation may be found where the lower molar mass peak is larger, which was claimed to be due to incomplete biosynthesis of rubber (Subramaniam 1993; referenced by Liengprayoon 2008). Tangpakdee *et al*. (1996) studied the number-average molar mass (M_n) of *H. brasiliensis* seedlings of different ages (1, 3, 7, 36 and 84 months) by GPC (SEC) and osmometry. The results led to the conclusion that M_n increased with the age of the tree. Parth *et al*. (2002 referenced by Liengprayoon 2008) observed that the peak area in the low molar mass region of RRIM600 decreased with age, thus favoring the peak area in the high molar mass region, while no distinct shift of the MMD_0 curves was observed. Although the shapes of distribution curves are different, the range of molar mass is approximately the same for all the clones (Eng and Tanaka 1993; referenced by Liengprayoon 2008).

The parameters provided by SEC and describing the MMD_0 curves are as following:

- Percentage of gel fraction (Gel)
- Average molar masses : M_n, M_w, M_z, M_{z+1} (in g/mole)
- The numbers of long chains and of short chains, proportional to the areas S1 and S2 respectively measured on SEC chromotograms
- Two calculated parameters:

- Polydispersity $I_p = M_w / M_n$, or the ratio between two average molar masses, which is a measure of the breadth of the molar mass distribution

- Ratio “R21” = S2 / (S1 + S2), or “Short Chain Weight Ratio” (SCWR). This ratio was suggested by F. Bonfils (Cirad) for characterizing the weight-share of the short chains in the total weight of the rubber samples in this study.

Among the four average molar mass parameters, only one, M_n or the “number-average molar mass”, has a clear physical meaning :

$$M_n = \Sigma Ni \times Mi / \Sigma Ni$$

Ni indicates the number of moles with the specific level Mi of molar mass. The indice i varies along the whole range of the distribution. M_n is the real average molar mass of all the chains in the rubber sample.

$$M_w = \Sigma Ni \times Mi^2 / \Sigma Ni \times Mi$$

As the denominator of M_w is the total weight of the sample, M_w is called “weight-average molar mass”.

The meaning of M_w can be clarified. SEC technique carries out a slicing of the rubber sample in a large number of small elements, by decreasing order of the chain sizes. Each element i is characterized by a number of moles Ni, a weight wi, and a molar mass Mi (with the relation wi = Ni x Mi). Two types of average molar masses can be calculated among these elements :

- average weighted on the number of moles Ni : $M_n = \Sigma Ni\ Mi / \Sigma Ni$
- average weighted on the weights wi : $M_w = \Sigma wi\ Mi / \Sigma wi$

The two other parameters M_z and M_{z+1} are “mathematical moments” of Mn, and therefore more abstract concepts:

$$M_z = \Sigma Ni \times Mi^3 / \Sigma Ni \times Mi^2 \text{ (called z-average molar mass)}$$

$$M_{z+1} = \Sigma Ni \times Mi^4 / \Sigma Ni \times Mi^3$$

The interest of M_w, M_z and M_{z+1} is that they reflect the importance of the relative weight of the long chains in a rubber sample. Logically, $M_n < M_w < M_z < M_{z+1}$. In practice, only M_n and Mw are considered, notably for the calculation of polydispersity (I_p).

It is aimed to introduce these traits into the breeding process. Studying the molar mass distribution of clonal native rubber requires only small quantities of latex (20 ml per sample), which is well adapted to early selection. In Genmap research, the two parents of the F1 family were contrasted for their molar mass distribution. PB217 (monomodal, type 3) can be clearly distinguished from many other clones, and notably from RRIM600 (bimodal, type 2).

1.3. Rubber genetics

1.3.1. Rubber breeding and selection

Rubber breeding has begun since 1876, when the transfer of plant material from Brasil to South-East Asia was achieved by Wickham and the British colonial administration (Dijkman 1951). For one century, this « Wickham » population has been almost the only source of genetic variability (Varghese, 1992; Clément-Demange *et al.*, 2007). In 1981, an international collection was organized by IRRDB (International Rubber Research and Development Board) in more than 60 Amazonian locations of Brazil, thereby providing the breeders with an enlarged genetic variability of the *Hevea brasiliensis* species. The new Amazonian populations were evaluated for genetic diversity (Chevallier 1988; Besse et al. 1994; Lekawipat et al. 2003a, 2003b; etc.) and for agricultural traits. The yield level of the wild Amazonian populations was comprised only between 10 % and 20 % of the level of the Wickham population, and the production of the best W x Am hybrids was still lower than the cultivated Wickham clones. As a result, whereas

the Amazonian populations were included in pre-breeding programmes for future use, the Wickham population is still the basic material for clonal selection in Asia.

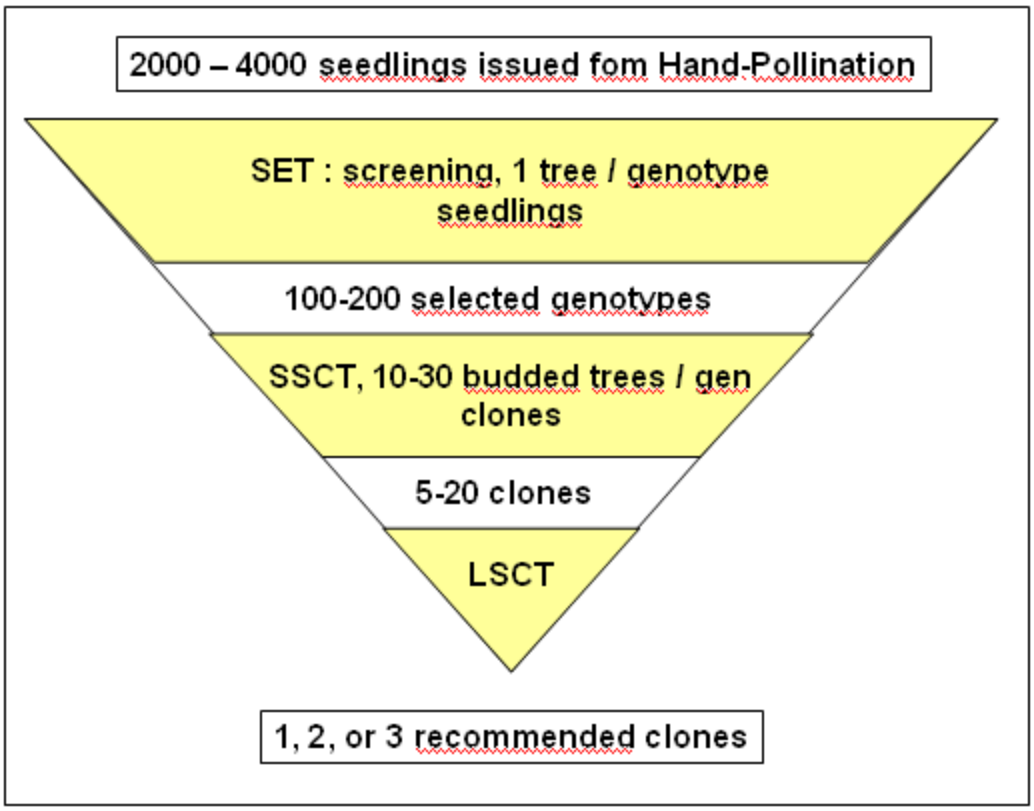
Rubber breeding, obviously conditioned by the characteristics of *Hevea brasiliensis* species, is mainly structured towards the objective of latex yield improvement, with many other traits contributing to this objective. Breeding for growth “before tapping” aims at early opening (first tapping), thereby reducing the duration of return on investment after planting. Growth “during tapping” is closely related to latex production itself. Selection is also applied to tolerance to Tapping Panel Dryness (TPD), wind damage, and leaf diseases (Colletotrichum, Corynespora, Oïdium, Corticium, etc.), and to adaptation to local environments. In Latin America, most of breeding efforts were devoted to resistance to the South American Leaf Blight due to *Microcyclus ulei* (SALB). New challenging objectives such as the quality of the rubber product, or rubberwood production, are emerging. Nowadays, the impressive development of biotechnologies holds promises, but their combination with conventional breeding in an integrated way is still a major challenge.

For this outcrossing species, the budding technique has been an important benefit as it makes possible the direct multiplication of the elites of highly heterozygous genotypes, at least for the aerial part of the tree. However the long duration of evaluation is an important drawback, which makes early selection a necessity. But as the rubber tree was never selected for seed production, its fecundity remained unchanged, and the low female fertility of most of the clones is a major constraint for recombination, thus limiting the diversity of crosses and strongly hampering the development of mating designs for the evaluation of parental effects (additivity and dominance, general and specific combining abilities). As a result, most of breeders’ efforts were focussed on the identification of elite genotypes within the families issued from hand pollination, rather than on the comparative study of the families and of the potential parents (Clément-Demange et al. 2007). Significantly, literature about family comparisons is very poor, apart from some methodological studies that were carried out in the 1970s’ for the estimation of the main genetic parameters (Gilbert et al. 1973; Tan et al. 1975).

Rubber breeding is organized in successive steps :

- Evaluation of germplasm and improvement of pre-breeding populations
- Choice of parents and recombination, mainly by hand pollination, with the creation of varied full-sib families (F1 families)
- Selection in three stages (figure 2) :
    - Seedling Evaluation Trial (SET), with high density planting, the evaluation of a large number (say 2,000) of different seedlings issued from hand-pollination and distributed over a number of families, screening among and within the families over a period of 2-4 years
    - Small Scale Clonal Trial (SSCT), with normal density planting and small clonal plots, evaluation of 100 to 200 clones issued from the selection in SET. Those clones can be distributed over different families for a combined family x individual selection. Evaluation lasts from 4 to 8 years and is carried out at the level of the tree potential
    - Large Scale Clonal Trial (LSCT), with normal density and large plots, with 6-20 clones issued from SSCT selection. Evaluation is carried out at the level of the stand, including susceptibility to wind damage and Tapping Panel Dryness over a long period of 15-20 years.
- Recommendation of clones and development.

Figure 2 : The three-stage clonal selection scheme in rubber breeding.



The three selection stages cover a period of 20-30 years with a progressive reduction in the number of selected genotypes and an increasing accuracy of the evaluation. SSCT, that combines the assessment of a reasonable number of genotypes with a good accuracy, is currently the core of the selection process.

According to Templeton (1969b), the current best clones, with annual yield little more than 2,500 kg of latex per hectare appear still far below a yield summit estimated to be around 9,500 lb/acre/year in the ninth year of tapping (nearly 12 T/ha). However, such an estimation may appear speculative and moreover unsustainable over the years. In Thailand there is a need for clonal diversification and increase in genetic performances. RRIT (Rubber Research Institute of Thailand) develops a rubber breeding programme for the joint production of latex and rubberwood.

In animal or plant breeding, the general objective is to increase genetic progress ΔG per time and per cost unit. This genetic progress depends on 4 factors :

ΔG = intensity of selection * accuracy of selection * genetic variability / generation interval.

In a tree crop like rubber, the generation interval is very long and can hardly be reduced. But early selection can reduce the duration from the beginning of selection to the release of new recommended clones. Therefore, early selection is a necessity. This is why the first and the second stages of selection are important. A major reduction of duration could be achieved if these two early selection stages could be merged. In the early selection of rubber, intensity of selection is already very high. Every year, we go from a few thousands of genotypes in SET to around one hundred clones studied in SSCT, and 5-10 clones studied in LSCT. Genetic variability depends on the variability between the families and the variability within each family. In the Wickham population of the rubber tree, variance within the families is larger than among the families. Therefore, we try to chose the best parents in order to create the best families, then selection is applied to individual genotypes within the families. Accuracy of selection is probably the point for which the possibilities of improvement are the most important, especially at very early stage, and notably if new genetic informations coming from molecular markers become available.

1.3.2. Early screening of seedlings in SET : a critical stage

In selection, phenotypic observations are used for inferring estimations of genotypic values. The quality of selection depends on the richness and the accuracy of the available information. In rubber, the first selection step (SET) is a critical phase. This stage is necessary for reducing the number of genotypes issued from hand pollination from around 2,000 to around 100 genotypes (5 %) that can be reasonably studied in SSCT. This means that around 95 % of the genotypes are discarded during this initial stage. By contrast, SET is the less accurate of the three selection stages. Therefore, any method able to increase the accuracy of SET selection with reasonable cost should be highly valuable.

The reasons why selection in SET is inaccurate, mainly for two reasons :

- Whereas budded clones are cultivated, SET selection is performed on seedlings. Although there is a positive correlation between seedlings and

their corresponding clones for production, it is not the case for growth. As a consequence, selection for growth fastness is normally impossible in SET.

- Whereas the accuracy of genetic value estimations is based on replications, a genotype is represented by only one tree in SET. Therefore it is not possible to distinguish genetic and environmental effects.

Important benefits might be drawn from tools able to provide new genetic informations independent from environmental effects, for selection at initial stage. There is a real hope that such information can be provided by Markers-Assisted Selection based on QTL knowledge.

1.3.3. Latex diagnostic and rubber breeding

Early selection for latex production is efficient for the identification of “quick starter” clones. But some very good clones such as PR107 or PB217 can exhibit their high production potential only after five or more tapping years. Latex diagnostic was introduced into SSCT for completing the selection of quick-starters with that of clones with a high potential in the long run (Odier 1983). The importance of latex sucrose content, as a predictor of yield potential in the long run, was shown on a set of clones, and explained by its importance for the production of energy and of the rubber chains (Tupy 1989; Gohet 1996). A high positive correlation between latex production and inorganic phosphorus (Pi) was shown, but Pi level was more a confirmation of the observed production than a prediction of the future. By contrast, in most cases, sucrose content (Suc) was observed as not related or even negatively related with latex production. A high initial sucrose level was found to confer a special ability for high production in the long run, a good response and tolerance to stimulation, and maybe also a good tolerance to TPD.

Latex diagnostic, as applied to young budded trees of 3–4 years old, early tapped with no ethephon stimulation in SSCT, allowed the classification of clones for their metabolic activity and the availability of sucrose in the latex. This method was used for selecting not only quick-starters but also slow-starters. Gohet (1996) discussed the methodology of latex diagnostic as applied to early selection.

1.3.4. From quantitative genetics to Markers-Assisted Selection

Quantitative genetics provides estimations of genetic values to be used in selection. The theory was initially developped by R.A. Fisher (1930) based on the assumption that many traits resulted from the combination of small effects issued from a large number of genes (polygenic traits). Quantitative genetics is a probabilist approach. Its capacity to use every available phenotypic information from the individuals and their relatives for distinguishing genetic and environmental effects and for optimising the estimations of genotypic values was recently emphasized by the fast improvement of calculation facilities. However, it addresses “invisible genes in a black box”. The development of molecular biology over the past two decades has led to a new phase in breeding where the genome and the genes have become accessible. In particular, the associations between molecular genetic markers and phenotypic traits (QTLs), appear directly oriented towards breeding through the potential application of Markers-Assisted Selection.

1.3.5. Biotechnologies and rubber research

Since 1918 (Van Helten), budding has been a major innovation in rubber, with the development of homogeneous clonal material, and a powerful technique for a fast use of genetic variability and the obtention of new performant cultivars. In vitro cultivation of rubber was presented by many authors and reviewed by Carron et al. (1989, 1995). This research was confronted with the question of « phase change » (juvenility/maturity, epigenetics). One objective is to develop new varietal types, more performant than the conventional clones. Somatic embryogenesis is also the pathway to genetic transformation (Arokiaraj and Wan Abdul Rahaman 1991 ; Montoro et al. 2003). Biochemistry research about the physiology of the laticifer system has led to the discovery of ethephon stimulation (Abraham 1968). Another success was the development of the « latex diagnostic » (Jacob et al. 1987) that can be used for monitoring the tapping systems and the intensity of stimulation, and for selecting « slow-starter » clones with high long-run production potential.

Molecular physiology was developed in rubber, predominantly on latex cells, on such thematics as rubber biosynthesis, latex cell functioning, latex coagulation process, ethylene biosynthesis and metabolism, oxidative stress, tapping cut dryness and brown bast, allergenic proteins in the latex, heterologous genes to be expressed in the latex, drought tolerance, leaf fungus diseases (*Corynespora*

cassiicola), cyanogenesis metabolism (research on SALB and TPD) or defence proteins, photosynthesis, and on an increasing number of other proteins. Large EST banks were created and sequenced for macro- or microarray-based studies of *Hevea* gene expression. Cloning of ethylene-inducible and/or laticifer-specific promoters from rubber tree has been undertaken (Pujade-Renaud et al. 2001). The *Hev2-1* promoter in transgenic lines was characterized in transgenic lines (Montoro et al. 2008), and new molecular constructs were prepared with this promoter, genes related to oxidative stress, and the GFP (Green-Fluorescent Protein) selection marker gene (Leclercq et al. 2010). These researches aim at understanding the metabolic pathways of the regulation of rubber production in response to the environment and technical practices. Sequencing of the whole rubber genome is now imminent.

After isozymes in the 80s' (Chevallier 1988), molecular genetic markers were developed at Cirad since the beginning of the 90s' and used for clonal identification (Besse et al. 1993), paternity testing (Blanc et al. 2001), and genetic diversity analysis (Seguin et al. 2003). Special efforts were devoted to the development of SSR markers or microsatellites (Seguin et al. 1997).

1.3.6. The QTL approach

Quantitative traits are assumed to be based on the expression of many genes with small effects. In fact, some of those genes may have large effects. A QTL is a locus (a segment of chromosome) harbouring a genetic factor significantly involved in the variation of a quantitative trait. Thus QTL detection is based on the identification of the associations between some genetic markers and some phenotypic effects, thereby showing the existence and localisation of important genes on the chromosomes.

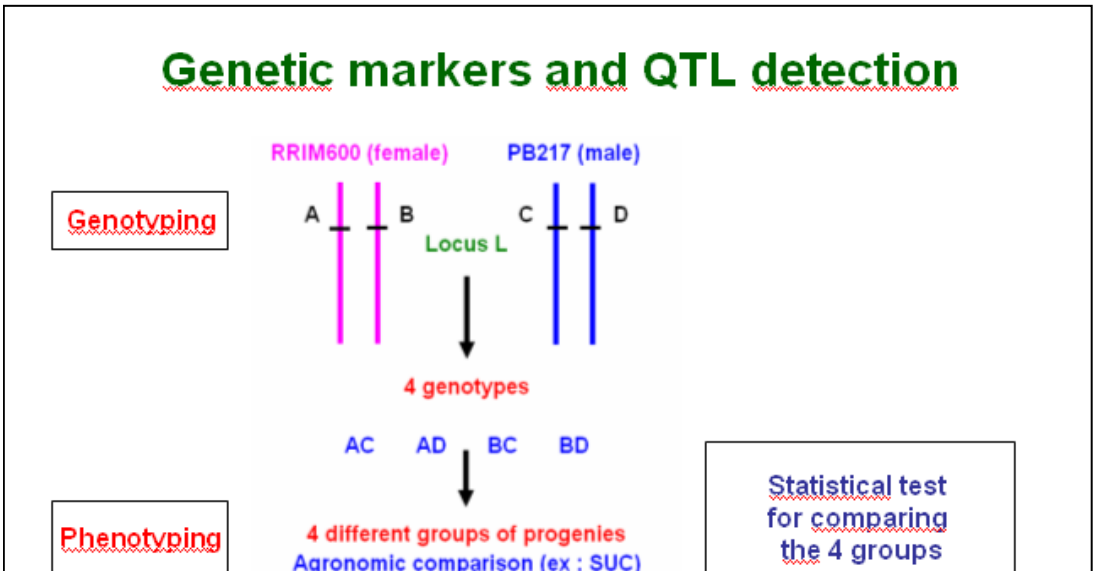
The QTL approach began to develop on a wide scale in the 80s', when the development of large numbers of molecular genetic markers became available, thanks to RFLP markers (Saiki et al. 1985), and PCR technology (Mullis et al. 1986). But the concept of genetic linkage was developed as soon as at the beginning of the 20th century (Morgan 1915), and a first illustration of the QTL approach was published by Sax in 1923. The first genetic maps were based on

morphological markers. Biometrical approaches have been proposed and used to study the associations between genetic markers and quantitative trait loci (Thoday 1961; Jayakar 1970; McMillan and Robertson 1974). Geldermann (1975) presented « a method using monogenic characters as markers for the mapping of effects having a share in quantitative traits, in natural or breeding populations ».

For one marker-locus with 2 alleles on one parent, the population of progenies of this parent can be subdivided into 2 classes, one for each allele. If one gene contributing to a quantitative trait is linked to the marker locus, and if the 2 alleles have differential effects, it is highly probable that the 2 classes differ for the trait. From this principle, with the use of many polymorphic markers, a lot of informations can be deducted on the genetic structure of the quantitative traits.

For illustrating it by a simple case, we can assume that one gene, important for the expression of a trait, is located on the same locus as that of a genetic marker (figure 3). In a F1 family (which is the case of the present research), the diploid genotypes of the genetic marker are « ab » for the female parent, and « cd » for the male parent (two alleles for each parent). Segregation in the progenies generates the four classes « ac », « ad », « bc », and « bd », with class sizes varying around the statistical expectation of the number of such classes. The trait is measured on the progenies, and the four classes are compared for this trait by statistical methods. If the four classes are significantly different between each other, it can be inferred that there is one gene important for the expression of the trait near the locus of the marker.

Figure 3 : Principle of QTL detection.



The first QTL approach in rubber was developed by Cirad (Lespinasse et al. 2000a, 2000b) with RFLP markers. It was then continued with SSR and AFLP. It was based on the family PB260 x RO38 for analysing resistance to South American Leaf Blight (SALB) caused by the fungus *Microcyclus ulei*. Phenotyping consisted in the observation of symptoms after artificial inoculation under controlled conditions. On the RO38 parental map, the main QTL, located on the linkage group g13, was detected for five different strains of *Microcyclus ulei*. For all the detected QTLs, the favourable allele (providing resistance) was inherited from F4542, the *H. benthamiana* parent of RO38, and none was inherited from the Wickham *H. brasiliensis* parent of RO38. On the PB260 parental map, only one QTL was identified on the linkage group g15, with the moderately pathogenic strain G70), and it explained only 9% of the variance of the trait. On the consensus map, the QTL analysis with the five strains allowed for the identification of six QTLs on the linkage groups g6, g10, g12, g13, g15, and g16. QTLs were detected on g10, g12, g13, and g15 in the same position as that observed on the RO38 map (Lespinasse et al. 2000b).

Le Guen et al. (2003) extended this phenotyping to field conditions in French Guiana. The major QTL located on linkage group g13 was detected again on the RO38 map, and it was responsible for 36 to 89% of the phenotypic variance of the studied resistance/susceptibility trait. Other minor QTLs were also detected (four in the RO38 map on the groups g2, g8, g13, and g14, and one in the PB260 map on the group g9). By continuing the study of other isolates on the same cross, Le Guen et al. (2007) found that only one QTL contributed to the partial resistance against a highly pathogenic isolate, and no QTL was detected for resistance

against the most pathogenic isolate. As an unexpected result, a single isolate could thus completely bypass this polygenic resistance. Enlargement of this research to « association genetics » through the study of « linkage desequilibrium » was carried out on some portions of chromosomes in Amazonian natural populations (Le Guen 2008). Research of new genetic factors of resistance to SALB is currently continued on other families.

1.4. Genotyping and the building of genetic linkage maps

Genotyping consists in the identification of the two alleles of each genetic marker locus for each individual of a population. Genetic mapping consists in analysing the segregations between non-homologous loci for positionning the loci on a map.

1.4.1. Genetic markers

A genetic marker is a locus on the genome for which the recognition of different alleles makes possible the distribution in classes of individual genotypes. Some genetic markers are multilocus, but monolocus markers provide more accurate information. Genetic markers can be morphological (shape of growth units, or leaves, etc.), biochemical (isozymes, proteins), or molecular (DNA fragments).

A “good” (fully informative) genetic marker must be:

- Polymorphic : Multiallelic on the studied population
- Codominant : All the different genotypes of the marker, homozygous and heterozygous, must be distinguishable among the individuals
- Non-epistatic : The genotype of the marker must be readable for one individual whatever the genotypes of the other loci
- Neutral : The marker has no influence on the phenotypic expression and is not submitted to selection pressure, which is important for population genetics. However QTL mapping is based on the links between markers and phenotypes, even sometimes with markers included inside the sequences of genes
- Not modified by the variation of the environment : The genotype of the marker can be read whatever the environment (which is different from physiological markers)
- Easy to read : However, one important limit of the use of genetic markers is the cost of genotyping a large number of individuals.

Molecular genetic markers (MGMs) are very numerous and distributed over the entire genome. Successive generations of MGMs were used, such as RFLPs or Restriction Fragment Length Polymorphism (Saiki et al. 1985), SSCP or Single Strand Conformational Polymorphism (Lekawipat et al. 2003b), CAPS or Cleaved Amplified Polymorphic Sequence (Tragoonrung et al. 1992), RAPD or Random Amplified Polymorphic DNA (Williams et al. 1990). One important innovation came from the PCR technique (Polymerase Chain Reaction; Mullis 1986) which, by use of couples of primers, allows the amplification of a given DNA fragment. Currently, the most important PCR-based markers, SSRs or microsatellites (Akkaya et al. 1992) have greatly enlarged the potential applications of MGMs, notably in rubber with paternity testing after natural pollination (Blanc et al. 2001), and for QTL mapping. In addition to these SSR markers in the Genmap research, AFLPs, which combine the use of restriction enzymes and PCR, were also used. SNPs (Single-Nucleotide Polymorphism) are a very promising new generation of markers (Tyagi

and Kramer 1996). A review (Vignal et al. 2002) examined the interest of SNPs for genetics, as compared to other molecular genetic markers.

SSRs are tandem repetitions of mono-, di-, tri-, or tetranucleotide units. The value of n (number of repetitions) varies and generates differences in length among many alleles (usually from 10 to 25) in a population. SSR markers are cheap for routine use, but their initial identification and development is costly. They are codominant and locus-specific. The high polymorphism of SSR markers is particularly interesting for genetic mapping of outbred populations such as tree crops, as most of these markers can be found heterozygous for the parents of a segregating progeny. The use of small gels in Licor DNA fragment analysers, with the visualization of the bands by fluorescence and automatic recording on digital pictures increased the genotyping capacity with SSR, and permitted to abandon the use of radio-elements. In rubber, development of SSR markers was reported by Seguin et al. (2001), Saha et al. (2005), Souza et al. (2009), and Le Guen et al. (2010).

AFLP technology combines PCR and elements of RFLP techniques (restriction enzymes). Polymorphism is issued from two different origins : differences of restriction sites, and of hybridization due to the specificity of the primers used in PCR. In only one experiment, many bands (from 50 to 100) can be distinctly observed, corresponding to different lengths and therefore to different DNA fragments and different loci. As a consequence each band is the allelic form of one different AFLP marker. In a F1 family, depending on the segregation pattern, an AFLP marker can be co-dominant or dominant. AFLP markers can be found in large numbers without any prerequisite on sequence information in the studied genome. Although they are less informative than SSRs (they are bi-allelic and generate only two marker-genotypes), the high number of markers observed in one sole experiment makes AFLP genotyping fast to develop on large populations. They can contribute efficiently to densify a genetic map, filling the “holes” between some couples of distant SSR markers on a linkage group (this was the case for Genmap).

1.4.2. Genotyping the progenies of a segregating population

During the genotyping phase, each marker is considered independently from the others. Genotyping begins with the selection of polymorphic markers on one or/and the other of the two parents of a F1 family, and is then extended to the whole progeny. Genotyping makes an important laboratory part of the work in a QTL mapping project, but high-throughput genotyping techniques are evolving very fast. The markers are observed from the bands of electrophoretic patterns.

For genotyping a F1 family by SSR markers, the number of genotypic classes per marker varies from 2 to 4, depending on the alleles in both parents. The possible segregation patterns are as following:

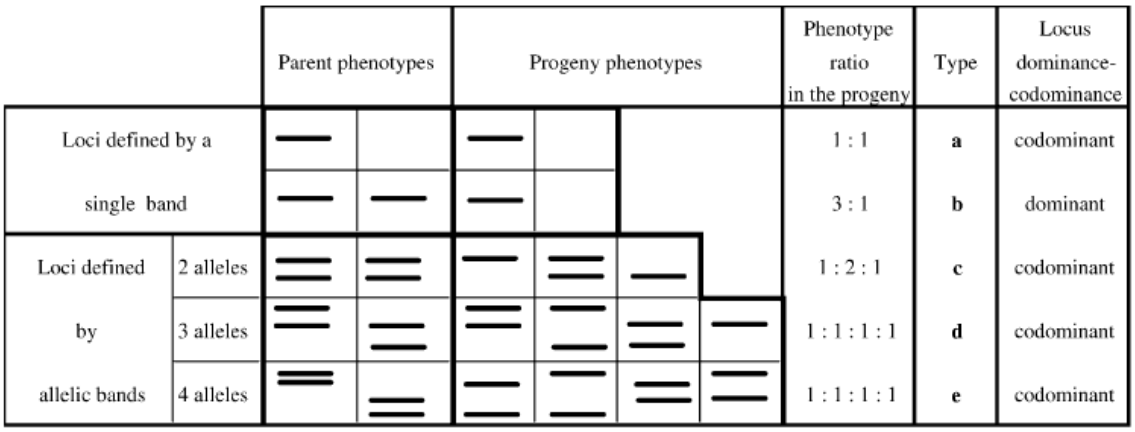
- <ab x cd> with 4 alleles and 4 genotypic classes “ac”, “ad”, “bc”, and “bd”
- <ef x eg> with 3 alleles and 4 classes “ee”, “ef”, “eg”, and “fg”
- <hk x hk> with 2 alleles and 3 classes “hh”, “hk”, and “kk”
- <ab x aa> (or <aa x ab>) with 2 alleles and 2 classes “aa” and “ab”

Figure 4 (Lespinasse et al. 2000a ; after Ritter et al. 1990) shows the five configurations of informative patterns in a F1 family. Two kinds of loci are presented : those defined by a single band (types a and b), for which only one segregating allele is observed (AFLP markers); and those defined by many allelic bands, for which the different segregating alleles are observed (SSR markers).

Correspondence of the 5 patterns with the cases of SSR and AFLP markers is presented hereafter:

- Type a : It corresponds, for AFLP markers, to segregation <ab x bb>, with allele “b” indicated by the absence of band. It fits also to the case of SSR markers with one parent homozygous for the allele “b”. In this case, reading is based only on the band corresponding to the allele “a”.
- Type b : It corresponds, for AFLP markers, to segregation <hk x hk>, with allele “h” indicated by the absence of band.
- Type c : It corresponds, for SSR markers, to segregation <hk x hk>.
- Type d : It corresponds, for SSR markers, to segregation <ef x eg>.
- Type e : It corresponds, for SSR markers, to segregation <ab x cd>.

Figure 4 : Informative patterns for mapping (Ritter et al. 1990). Any segregation found in a F1 family can refer to one of these five configurations.



Codominant loci such as RFLP, isozymes or microsatellites generate a larger number of segregating classes and they are more informative than dominant loci. Markers with 3 or 4 alleles are more informative: they allow the estimation of recombination rates (related with the genetic distance between 2 loci) with the best accuracy. Those markers are also more powerful for QTL detection, provided that the number of individuals is high enough, because they generate 4 classes of individuals in the progenies that can be more probably significantly different from each other. For AFLP, only 2 classes can be observable.

1.4.3. Genetic mapping

Genetic mapping involves the study of the markers by couples for ordering and positionning them on the genome, based on the analysis of segregation through meiosis and the estimation of recombination rates.

During meiosis, one chromosome in each pair is taken at random for designing the haploid genome of one gamete (inter-chromosomic recombination). Two loci of two non-homologous chromosomes segregate independently, with a recombination rate of 50 %. By contrast, two loci on one same chromosome can be independant or linked, depending on their proximity and their recombination rate. During the pairing of homologous chromosomes, crossing-overs generate the exchange of chromosomic arms (intra-chromosomic recombination). When a crossing-over occurs between two loci, there is a change in the association of the alleles of these

loci. The probability of crossing-over increases with the distance between two loci. There is a linkage when the recombination rate is lower than 50 %. Chromosomes are obviously the largest units of linkage groups. In only one generation change, most of the links between loci are maintained. The use of segregating families issued from a minimum number of generations (such as, in outbred species, a F1 full-sib family issued from one generation of recombination) appears to be optimum for estimating short genetic distances between pairs of loci, which correspond to low recombination frequencies.

A genetic linkage map is said « saturated » when any locus is linked with at least one neighbouring marker (independence begins with a recombination rate higher than 0.50). Step by step, linkage groups are constituted, and the number of linkage groups is reduced. In a saturated linkage map, the number of independent linkage groups is equivalent to the number of chromosomes of the haploid genome.

In the two parents of a F1 family, any of the four homologous chromosomes bears an original arrangement of alleles at its different loci, that represents the initial “phase”. In one genotype of the progeny, two alleles coming from one same chromosome of a parent, in the absence of recombination, are “in coupling”. By contrast, two alleles coming from the two distinct homologous chromosomes of one parent, after recombination, are “in repulsion”. In genetic mapping, for every couple of loci, segregation analysis leads to the identification of the non-recombined (coupling) and recombined (repulsion) gametic cases, thus allowing the estimation of recombination rates. For determining the phases, a standard phase is chosen for one locus on each chromosome, and this reference is used for characterizing the phases at the other loci. An illustration is given hereafter for two loci Li and Lj :

Locus Li : alleles a, b, c, d	segregation <ab x cd>	phase (0; 0)
Locus Lj : alleles e, f, g, h	segregation <ef x gh>	phase (1; 0)

When found in one same genotype of the progeny, the non-recombined couples of alleles (a, f), (b, e), (c, g), and (d, h) are “in coupling”; the recombined couples of alleles (a, e), (b, f), (c, h), and (d, g) are “in repulsion”.

Due to multiple crossing-overs between distant loci, and to the « interference » phenomenon (two crossing-overs between very close loci), the relationship between the recombination rate and the distance is not linear. Recombination rates are not additive, and have to be converted into additive distances for genetic mapping. The Haldane distance does not take into account the interference phenomenon : a recombination between two loci is not detected if there were an even number of recombinations. The Kosambi distance includes a parameter which takes into account the interference phenomenon. The unit of these genetic distances is the centimorgan (cM). A statistical test is required for declaring a linkage between two loci. The χ^2 is sometimes used, but more often the LOD score, which is derived from the Maximum Likelihood statistical method. Due to the large number of tests made for building a genetic map, and for reducing the number of linkages significant just by chance, an adapted LOD threshold has to be determined.

The double pseudo-testcross (Grattapaglia and Sederoff, 1994) is the most common method to create linkage maps of F1 families in outbreeding species. The principle is to consider the cross between two heterozygous parents as 2 testcrosses : the testcross of one parent onto the other, and vice-versa. Thereby, one map is built for each parent. Then a consensus map between the two parental maps is established by use of common « bridge » markers. Mapmaker (Lander et al. 1987) is a popular software for genetic mapping. The software Joinmap (Stam 1993) can integrate the information from several maps and construct a composite map from several populations. Joinmap also makes it possible to analyse populations from crosses between heterozygous genotypes.

The development of RFLP markers (Saiki et al. 1985) made possible the construction of the first dense genetic linkage maps. Two of them were published for maize and tomato as soon as in 1986 (Helentjaris et al.). In tree crops, genetic maps were initially published for fir (Tulsieram et al. 1992), eucalyptus (Grattapablia and Sederoff 1994), poplar (Bradshaw et al. 1994), and cocoa (Lanaud et al. 1995). The first genetic map for *Hevea* spp. (2n=36) was published by Lespinasse *et al.,* (2000a).

1.4.4. Maximum likelihood and LOD score methods

The Maximum Likelihood (ML) method is different from the Least Square (LS) method. The LS method (published by Gauss in 1809) is used for estimating the parameters of a mathematical model (a function) from a series of experimental data. The best function is that which minimizes the sum of squares of the deviations of the data from the predictions of the function. Minimization if achieved through a derivation of the function with respect to the parameters. A simple application is the estimation of a regression equation from a set of couples of data.

The ML method (developed by Fisher around 1920) is a statistical method for fitting a statistical model to data, and providing estimates for the model's parameters. For a sample of data X, the likelihood function $L(\theta|X)$ of a parameter θ given the data X is proportional to the probability of obtaining the data X given θ. The ML estimate of θ is the value of θ that maximises $L(\theta|X)$, obtained by derivating L with respect to θ. Therefore, the ML method provides estimates for the model's unknown parameters. In other words, for a certain set of data, maximum likelihood finds the values of the model parameters that make the data "more likely" than any other values of the parameters would do. The ML method is efficient for estimating variance components in models with unbalanced data, notably in mixed models. It is used in genetic mapping and QTL mapping for estimating the recombination rates and the LOD score statistics.

For genetic mapping, ML is used for the estimation of the recombination rate between two loci. Considering a segregating population, the progenies are distributed in different classes of recombination depending on the parental alleles. The likelihood function fitted to this case is a multinomial law. The sizes of the observed classes are assumed to be the most probable, so that the derivative of the likelihood function is null. It follows an estimation of the recombination rate « r1 ».

For testing the significance of the linkage between two markers, one can use a χ^2 test in a simple approach, by comparing the observed distribution of the sizes of the segregating classes with the theoretical distribution, based on the « nul

hypothesis » of the absence of linkage. Another approach uses the calculation of the LOD score, introduced by Barnard (1949), which is associated with the ML method. With the hypothesis H1 of a linkage between the two markers, the maximum likelihood L1, corresponding to the estimated recombination rate « r1 », is calculated. With the hypothesis H0 of the absence of linkage, the likelihood L0 is calculated for r = 0.5.

$$\text{LOD score} = \log_{10} (L1/L0)$$

1.5. Phenotyping

Phenotyping means measuring a trait which is submitted to genetic and environmental influences. Breeding and selection are based on the observation of phenotypic traits for estimating the genetic values of these traits (“from phenotype to genotype”). Phenotypic data analysis will also allow one to estimate genetic parameters such as the variances of the genetic and environmental effects, the heritabilities of the traits, and the correlations between the traits. In QTL-mapping, phenotyping includes the implementation of an experiment for the study of the segregating progenies of the chosen population, the measurements of the traits, and the estimation of the genetic values which are to be used together with the genotypic data for QTL detection. Higher the accuracy of genetic value estimations, and higher the power of QTL detection.

1.5.1. Distributions of the data

The statistical linear model is a powerful analysis procedure able to distinguish significantly different levels of treatments. But using this model is possible only with normally distributed data (although strict normality is not required). Statistical softwares provide many hypothesis tests for normality (Kolmogorov-Smirnov, Shapiro-Wilk, etc.). Data transformations (log, root, etc.) can sometimes modify the original distribution and makes it normal. If no data transformation gives satisfactory result, the data should be analysed by non-parametric statistics (such as Kruskal-Wallis test) that are less powerful than linear models.

In rubber, growth traits are generally normally distributed. But the distributions of rubber production among the progenies of a full-sib family, such as observed in Seedling Evaluation Trials (SET), is not normally distributed but strongly dissymetric towards lower values («rightly skewed »). By itself, this dissymetric distribution is triggering, and suggests the possibility of some specific genetic determinism of rubber production.

1.5.2. Experimental design and statistical model

The power of QTL detection is highly dependent on the quality of phenotyping. The accuracy of the measurement is of course important. Another important aspect is related with the experimental design which is aimed at controlling the variations of the environment within the trial and increasing the accuracy of the estimation of genetic values. One first principle is to observe each genotype many times in different parts of the trial (replications). It follows a need for structuring the trial into homogeneous subsets (blocks) and for distributing the plots among the blocks (one plot is an elementary unit corresponding to one replication of one genotype).

In rubber, the genetic heterogeneity of rootstocks, from one tree to the other, is part of the environmental variation. For reducing this source of variation, it can be advised to plant more than one budded tree in a plot, and to use the average of the individual tree-measurements for characterising the phenotypic value of each plot.

A variety trial often has a large number of levels of treatment (varieties themselves) and a small number of replications. As a consequence, the total area of the trial is large. If a randomized complete block design is set, there will be an important environmental variation between the treatments of one same blocks, thus increasing the error variance and reducing the power of the experiment. It results in a reduction in the heritability of the traits. Therefore, incomplete block designs are used, where only small subsets of varieties are put together in small incomplete blocks, thereby reducing the risk of environmental heterogeneity within each block. However a compromise is to be found because, with many small blocks, there is also a risk to generate block x treatment interaction.

Incomplete block designs may be balanced, or only partially balanced. A design is balanced if all the pairs of treatments appear in the same number of blocks. As a consequence, all the treatments are compared by pairs with the same precision. The different pairs of treatments may appear in zero, one, two, three, or more blocks, each number being called an « association class » of the corresponding subset of pairs of treatment.

For generating partially balanced incomplete block designs with satisfactory statistical properties, varied softwares were created. A cyclical way for distributing the treatments in the blocks is often used, due to the easiness and flexibility of this approach. Among them, the « α-design » (Patterson and Williams, 1976), is particularly well adapted to the comparison of a large number of treatments. It allows the construction of resolvable incomplete block designs with any number of varieties (v) and block sizes (k) such that v is a multiple of k. The blocks must be capable of arrangements in complete replications. This condition is a pre-requisite for the construction of resolvable designs. All the varieties have the same number of replications. Resolvable designs have many advantages. They make possible the partial phenotyping of the trial on a restricted number of replications for the most expensive measurements. They can also be analysed in the form of complete block designs if it is proved profitable for some traits. In such designs, not all the pairs of varieties appear in at least one block, but in some cases, some pairs may appear in two blocks or even more.

The 4 parameters of an α-design are :

- v : number of varieties or genotypes
- r : number of replications
- k : number of varieties per incomplete block
- s : number of incomplete blocks per full replication.

… with the condition : $v = k.s$

It was found that if s is an odd number and not a multiple of 3, and k lower or equal to s, then all the possible pairs of varieties are in no block (0) or only one block (1). These designs with only two association classes, called α (0, 1), are the most efficient. Elsewhere, if k is lower or equal to s^2, then all the possible pairs of

varieties are in no block, or in one block, or in two different blocks ; these designs with three association classes are α (0, 1, 2) designs.

With this design, the statistical model is :

$$Y_{ijk} = \mu + G_i + b1_j + b2_{k/j} + G_i \times b2_{k/j} + \varepsilon_{ijk}$$

G_i : random effect of the genotypes
$b1_j$: fixed effect of the complete blocks (replications)
$b2_{k/j}$: fixed effect of incomplete sub-blocks nested in the complete blocks
$G_i \times b2_{k/j}$: random effect of the interaction genotype x sub-block
ε_{ijk} : random effect of the experimental errors.

Most of agricultural experiments are made of fixed effects. A fixed effect is a treatment with specific levels (for example: three levels of a fertilizer, or five varieties). By contrast, a random effect is a treatment with a set of levels that were taken at random in a population. Experiments with random effects are often developed in plant breeding for assessing genetic variance in a population. In mixed models, there is a mixture of fixed effects and random effects. Mixed models make possible optimized estimations of genetic values (Henderson 1984) that are called “BLUPs” (Best Linear Unbiased Predictors). Those models are particularly useful for the analysis of unbalanced designs. Furlani et al. (2005) presented one application of a mixed model to genetic estimations in rubber.

1.5.3. Heritability

Heritability is the proportion of genetic variance in the overall phenotypic variance of a quantitative trait measured over a population of plants and genotypes. Narrow sense heritability h^2n is restricted to the proportion of « additive » genetic variance in the phenotypic variance. Within a F1 family, the distinction between additive variance and dominance variance is not possible, and therefore, only the broad sense heritability h^2l, indicative of the proportion of total genetic variance in the phenotypic variance, can be estimated.

For a plant « j » representing a genotype « i », its phenotypic effect can be described by an additive linear model :

$$Pij = \mu + Gi + \varepsilon ij$$

The effects P, G and ε of this model are random effects, with Cov (G, ε) = 0. In such a case, the relation between the three variances VP, VG, and Vε is also additive :

$$VP = VG + V\varepsilon$$
$$h^2 = VG/VP = VG \ / (VG + V\varepsilon)$$

This heritability h^2 is based on the phenotypic variance of individual plants. It is called heritability « at the individual plant level ». By contrast, we can modelize the phenotypic value of the mean of n plants representing one genotype Gi :

$$Pi. = (\Sigma Pij)/n = n\mu/n + nGi/n + (\Sigma \varepsilon ij)/n$$
$$= \mu + Gi + (\Sigma \varepsilon ij)/n$$

The phenotypic variance of Pi. is :

$$VPg = VPi.$$
$$= VG + 1/n^2 \times \Sigma V\varepsilon$$
$$= VG + 1/n^2 \times (n\ V\varepsilon)$$
$$= VG + V\varepsilon/n$$

From this phenotypic variance, another heritability expression H can be deducted : the heritability « at the level of the genotype mean ». Its expression is :

$$H = VG / VPg = VG / (VG + V\varepsilon/n)$$

Of course, H is higher or equal to h^2. With a mixed model and unequal numbers of trees per plot and of replications per genotype, no fully satisfactory solution was found for the calculation of H. Therefore, it seems preferable to estimate h^2 heritabilities at individual levels (and not H) in such cases. Heritability estimates (h^2

or H) are highly dependent on the genetic population, the heterogeneity of the local environment of a trial, and the experimental design. The main interest of heritability estimates is to compare their values between different traits in one same experimental design and one same statistical model.

1.5.4. Correlations between traits

A correlation can result from both environmental or/and genetic origins. In a heterogeneous trial, trees growing in fertile soil will be bigger than those growing in poor soil, and girth and height will be positively correlated (environmental correlation). A genetic correlation can be due to a pleiotropic gene, or to the global effect of a set of genes involved in the expression of the two traits (with a common genetic cause underlying the expression of both traits). A genetic correlation may be due also to the direct influence of one trait on the other (causal reason).

In rubber, bigger the tree and higher the latex production. In commercial plantations, only the trees reaching a standard girth (usually 50 cm) are opened for tapping, and successive openings are carried out along time until all the trees are opened. As a consequence, the homogeneity of the size of the trees is very good. By contrast, in small scale clonal trials (SSCT) such as in Genmap trial, all the trees higher than a minimum girth (25 cm) must be opened all at the same time. It follows that the girths of the trees vary a lot, and tapping cuts do not have the same length. Therefore, the comparison of the genotypes for rubber production must take into account the sizes of the trees. One method of correction is integrated with BLUP estimations by putting the girth as a covariable, as one more fixed effect in the statistical model of rubber production traits. This is equivalent to adjusting all the production data to one same mean girth of all the trees and genotypes, so that the BLUP estimations of production are independant from the girths of the genotypes. In another approach, the corrections can be made after BLUP estimations, by calculating the regression of one trait on the other correlated trait, at the level of the BLUPs.

1.6. QTL mapping

1.6.1. General aspects

Like genetic mapping, QTL mapping is based on the observation of the links between couples of neighbouring loci, and more precisely between the loci of “invisible” genes and the loci of their closest “visible” markers, through the analysis of segregation from the parents to the progeny. Whereas the markers can be observed through genotyping, the genes can be detected through their effects on the phenotypes. QTL localization is statistically based and only approximate.

One must distinguish the locus of a QTL and the loci of its neighbouring markers (with the exception of a marker located inside a gene). Due to the origin of the parents, two alleles of a marker may be associated to one same allele of a QTL (monomorphic QTL) ; conversely, two alleles of a QTL may be associated to one same allele of a marker (monomorphic marker) ; such QTLs are undetectable. Actually, one QTL-mapping experiment can detect only a fraction of all the QTLs in a population. Limitations come from the chosen family, the genetic composition of the parents, the size of the experimental population, the density of the mapped markers, and the more or less regular distribution of the markers on the genetic map.

For a given experimental system, one main interest of QTL detection is that it priorily reveals the activities of some of the most important among the genes contributing to the expression of a quantitative trait. Moreover, as far as an experimental population has been genotyped and mapped, it can be used for QTL detection associated to any easily measurable trait, and thus for a wide range of applications. When two QTLs are detected for two different traits on the same position, there may be only one QTL with pleiotropic effect, or two genetically independant QTLs, but linked on the same chromosome segment. Variations in the environment modify the expressions of genes and therefore the effects of the QTLs. For these reasons, it is generally useful to search for QTLs on more than one family and one environment.

The QTL approach includes « QTL detection » and « QTL mapping ». A QTL can be detected based on its genetic effect, and then located as precisely as possible on the genetic map. A QTL is characterized by its estimated genetic effect and its most probable position. Genotyping and phenotyping of the progeny of a family are a necessity for QTL detection. Whereas the genetic map results only from the analysis of genotypic data, QTL mapping (detection and localisation) results from the combined analysis of genotypic and phenotypic data.

The effect of a QTL, « %exp », is expressed in « percentage of the total phenotypic variance ». From this point of view, « %exp » can be considered as a form of heritability of the QTL. In QTL detection by variance analysis, « %exp » is equivalent to the coefficient of determination R^2, calculated as the ratio of the sums of squares between the QTL and the total variance. Although the BLUPs are optimized estimators of the genetic values, they must be considered as phenotypic values, and the phenotypic variance is the variance between the BLUPs.

1.6.2. Experimental populations and estimation of the effects of a QTL

QTL detection is based on segregation analysis in a population. Many types of experimental populations can be studied for QTL detection such as :

In autogamous plants :

- F2 family. A non-segregating F1 family is issued from the cross of two pure lines. Then F1 individuals are selfed for generating a segregating F2 family. There are two alleles at each polymorphic locus in the family, and three possible genotypes : aa, ab, and bb (homozygous and heterozygous).

- A population of Recombined Isogenic Lines (RIL) issued from several generations of selfing. There are two alleles at each polymorphic locus in the family. Due to the many recombination events (many generations of recombination), the genetic distances on the map are different from that on the map of a F2 family. There are two possible homozygous genotypes at each locus.

- A population of « Doubled-Haploid » lines (DH). In this case the genetic map is similar to that of a F2 family (one generation of recombination) but the individual progenies are homozygous pure lines, with two possible homozygous genotypes at each locus.

In outbred species :

- A segregating F1 family issued from the cross of two heterozygous parents (« CP » populations). There may be a maximum of four alleles and four heterozygous genotypes at each polymorphic locus in the family. In this case, two parental maps and a consensus map are built.

In animal breeding (often based on selection of male parents) :

- A half-sib family (one male is sibed with many females). Segregation analysis takes into account only the two alleles issued from the male parent at each polymorphic locus. In this case one map of the male parent is built.

For the F2 populations issued from inbred lines, and for one segregating codominant marker (<ab x ab>), the three genotypes « aa », « ab », and « bb » can be observed and the values of additive effect (a) and dominance effect (d) of the associated QTL can be estimated. For F1 populations issued from heterozygous parents, segregation can concern up to 4 alleles (<ab x cd >) and generate the four genotypic classes « ac », « ad », « bc », and « bd ». The effects of individual alleles cannot be estimated because no homozygous genotype can be observed, and each of the four classes includes a mix of additivity and dominance. However three genetic effects (female, male, and interaction) can be tested in a two-way factorial design (with two parental alleles as two levels for each of the two treatments, female and male), by determining the genotype class of the QTL for each progeny in an approximate way, based on the information from neighbouring markers. If a significant interaction is detected, it can be inferred that the hypothesis of absence of dominance is wrong. If no interaction is detected, the respective contributions of the female and of the male alleles to the QTL effect can be compared and discussed.

1.6.3. Linkage desequilibrium

Another type of research, not investigated here, concerns the analysis of « linkage desequilibrium » (LD) at the level of wider populations issued, after many generations from a set of original parents. This can be seen as an extension of the study of linkage and recombination, based not only on the physical proximity between two loci but also on the variation of crossing-over frequencies along the genome, and on the structure of the population resulting from the history of the recombinations between its members. Such a study is no more limited to the minimum number of 2, 3 or 4 alleles per locus in a segregating experimental family, but it has to take into account all the alleles of the population for each locus.

Whereas the association of two loci on a chromosome is studied with limited recombination between them in a segregating family, LD describes a wider situation in which some combinations of alleles occur more or less frequently in a population than would be expected from a random formation of gametes based on the frequencies of these alleles. Numerically, LD is detected when the frequency of joint detection of two alleles on two different loci is different from the product of the frequencies of each allele (deviation from random association). In population genetics, LD can be observed even between two loci which are not on the same chromosome. Non-random associations between pairs of loci are measured by the degree of LD in a population, and LD studies aim at determining which fraction of LD can be attributed to the physical proximity between loci or to the genetic structure of the population.

In a population with no LD, the association between a marker-allele and a QTL-allele detected in the progenies of one experimental family cannot be assumed to exist also in other families of the same population. By contrast, in a population with a high degree of LD, one favorable association may have a higher probability than random to be found among the members of the whole population. These aspects generate different strategies of Markers-Assisted Selection.

1.6.4. QTL detection by « single-marker » method

Genotypic information at a marker position is perfectly known. In such a simple case, testing the presence of a QTL at a marker-locus can be carried out by a one-way variance analysis, with the following statistical model :

$$Y_{ij} = \mu + M_i + \varepsilon_{ij}$$

Y_{ij} represents the phenotypic value of the progeny j for the marker, and M_i represents the random effect of the segregating class i (de Vienne 2003).

QTL detection on single markers is robust to deviation of trait phenotypic values from normality but not much powerful. In general, QTLs are not located exactly on marker-loci. Some methods were developed for detecting a QTL near one marker. However, the power of such methods decreases with the distance between the tested position of the putative QTL and the marker. Therefore a high denstity of markers on the map is required for increasing the efficiency of these methods.

1.6.5. QTL detection by « Interval Mapping » method

« Interval Mapping » method (IM), a « two-marker locus » method, was developed by Lander and Botstein (1989), detailed by Van Ooijen (1992a), and included in the software MapQTL5 (Van Ooijen 2004). It is currently the most popular approach for QTL mapping in experimental populations, and has now become a current standard method. It is based on the calculation of a LOD score. It is a « single-QTL » model, based on the hypothesis that there is only one (or no) QTL in the interval between two linked markers. Every position on the genome is tested for the presence of a QTL, with a certain distance between two successive positions (e.g. every 1 cM). The two markers flanking each tested position are used simultaneously, which gives a maximal linkage information for a position on a chromosome.

The statistical aspect of IM is worth to be described. « Interval Mapping » is a maximum likelihood approach to the segregation of a « mixture of probability distributions » (Van Ooijen 2004). The method is here explained in the case of a F1 family with 4 possible classes of genotypes at any genome position. For a

phenotyped trait, each of the N progenies of the family has a phenotypic value x. There is a normal distribution of these N phenotypic values.

We want to test a QTL position X located between 2 flanking markers A and B. The distance between the two markers is given by the recombination rate « r ». The recombination rates between A and X, and between X and B, are « ra » and « rb ». We consider that the tested QTL position is given by « ra ».

The combined genotypic information of the two markers, which is supposed to be perfectly known, determines a maximum number of 4 x 4 = 16 marker classes. The unknown genotypic information of the QTL position determines a number Q = 4 QTL classes.

Considering one marker class m, each progeny of this marker class belongs to one or another of the 4 QTL classes (class q of the QTL). Therefore, the progenies of the marker class m make a mixture of 4 distributions (one for each QTL class) with four means μ1, μ2, μ3, and μ4 (unknown), and with the same residual variance σ_r^2. For one progeny, the phenotypic value x, belonging to the unknown distribution q, is associated with a probability density function (pdf) : fq(x).

The probability of the QTL class q depends on the marker class and on the QTL position determined by « ra ». This probability is : π_{mq}. We can note that the sum of the 4 mixing proportions is equal to 1 :

$$\pi_{m1} + \pi_{m2} + \pi_{m3} + \pi_{m4} = 1$$

If, for instance, the QTL position coincides with a marker, and the marker genotype of the individual is completely known, then one of the Q component probabilities π_{mq} equals 1 while the others are 0.

The phenotypic value x of a progeny has the probability density function :

$$pdf = f(x \mid m ; ra) = \Sigma_q \pi_{mq} . fq(x) \text{ , with } \Sigma_q \text{ from 1 to 4}$$

This pdf is conditionned by m and « ra ». The summation Σ_q is done over the 4 QTL classes, from 1 to 4.

For each marker class, there would be a separate mixture model. But, since these models have the same components fq(x), and the mixing proportions are related through the linkage map, one general model can be specified.

If the position tested for the presence of a QTL is between two markers while the genotypes of these flanking markers are not completely known (lacking data, or dominant AFLP markers), then the genotypes of the linked markers beyond the flanking markers and their map positions are used to obtain the probabilities. Thus, genetic information from the markers surrounding the tested position is used to calculate the most accurate values of the component probabilities π_{mq} , also called the « mixing proportions » of the model.

The likelihood function L1, or joint probability density function of the entire family, under the hypothesis that a QTL is segregating, is :

$$L_1 = \Pi \Sigma \pi_{mq} . f_q(x_k)$$

with Π being the product from 1 to N (with N progenies in the family). All the unknown parameters in L1 make a vector θ. Maximization of L1 is performed by deriving L1 on θ : δ(L1) / δ θ, by use of an « EM algorithm » (E, expectation ; M, maximization ; Dempster et al. 1977). This algorithm is an iterative procedure which, at each iteration, calculates the logarithm of the likelihood. The iterations stop when the log-likelihood function has become smaller than the so-called « functional tolerance value », which means that the algorithm has converged.

The maximization of L1 allows the estimation of the genetic values of the trait for each QTL genotype (means μ1, μ2, μ3, and μ4).

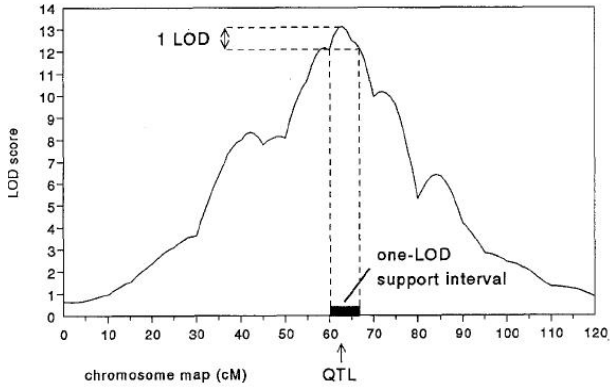
The likelihood L_0 under the null-hypothesis (no QTL segregating) is similar to L_1 except that there is just a single component in the mixture (only one genotypic class for the studied position). The comparison of L_1 and L_0 is done by using the

LOD score method (the calculated LOD score is compared to a significance threshold) :

$$LOD = {}^{10}\log (L_1 / L_0)$$

In MapQTL5, the whole genome is scanned for successive positions at regular distances (say every A cM). If a marker is met along the scanning at a distance from the preceding marker lower than 1 cM, this marker becomes the next tested position. The LOD score is calculated for every position, and a QTL likelihood map is constructed by plotting the LOD score against the genome map. Such a map can be regarded as the likelihood profile for the position of a QTL, although in principle it is a connected series of likelihood profiles for chromosome segments between two neighbouring markers. The fact that LOD scores for neighbouring segments are calculated with one shared marker and one marker different, results in a curve that is angled at the marker locations. The maximum likelihood estimator of the position of the QTL is the point on the map for which the curve has its maximum. To obtain a sort of a confidence interval of the position of the QTL, a so-called « one-LOD support interval » is constructed by taking the two positions, left and right of the point estimate of the QTL, that have a LOD score of one less than the maximum (figure 5). One LOD less corresponds to a probability of a factor ten less than the most likely position (Van Ooijen 1992)

Figure 5 : Example of a QTL likelihood map. The construction of a one-LOD support interval is demonstrated (from Van Ooijen 1992).



In practice, there will always be individuals for which the marker genotype cannot be determined (lacking genotypic data, or undetermined data in the case of some AFLP markers). In such cases, the likelihood contribution of an individual for a given position in the genome is based upon the closest known flanking markers (with the limit of 5 successive markers in MapQTL5).

With the IM method, Paterson et al. (1991) investigated the effect of the environment on QTL expression. Only four detected genes were expressed in the three environments studied, while 25 others were expressed in two or one environment. This might indeed reflect genotype x environment interaction.

As compared to single-marker methods, IM is more accurate for intervals larger than 20 cM (Rebai et al 1995). The accuracy of IM method was evaluated by simulation (Van Ooijen 1992). With a minimum population of 200 backcross or F2 individuals, there is a reasonable probability of detecting QTLs explaining at least 5 % of the total variance. The number of individuals and the relative size of the genotypic effect of the QTL are important factors determining the mapping precision. In average, a QTL with 5 % or 10 % explained variance is mapped on an interval of 40 or 20 cM respectively. QTLs with a larger genotypic effect will be located more precisely. The precision of QTL mapping, disappointing for gene cloning purpose, is adequate for breeding purpose.

However, IM method has some limitations :

- Its powerness is limited by the fact that each locus is assessed as a putative QTL position independantly from other QTLs on the genome. Testing of « one QTL » at an interval is carried out against the hypothesis of no QTL on the whole genome. Thereby the variance due to other QTLs is mixed with the residual variance, thus reducing the power of detection.

- It does not allow precise detection of nearby QTLs on the same chromosome. Thus, when two neighbouring QTLs act in the same direction (coupling), there is a risk of detecting a single « ghost » QTL between the two real ones (Martinez and Curnow 1992). If the two QTLs are far enough

away (more than 50 cM), it is easier to estimate the effect and the position of the second by « fixing » the position of the first, as suggested by Lander and Botstein (1989). In most cases, two neighbouring QTLs with effects in repulsion may be ignored. More generally, it can be said that IM is weak for the detection of multiple QTLs on one same chromosome.

1.6.6. QTL detection by « approximate Multiple QTL Mapping »

A large number of factors influence the accuracy of QTL mapping. Single-marker and IM approaches are biased when multiple QTLs are linked to the marker interval being considered. Marker-trait associations can be assessed using one-, two-, or multiple-marker locus genotypes. Under a single-marker analysis, the distribution of trait values is examined separately for each marker-locus. In this case, each marker-trait association test is performed independantly from all other markers. Methods simultaneously using three or more marker loci attempt to reduce or remove bias (multiple-marker methods). Multipoint mapping (Wu and Li 1996) considers all of the linked markers on a chromosome simultaneously, resulting in a single analysis for each chromosome (Lynch and Walsh 1998). Theoretically, a multiple-QTL approach based on simultaneous multiple regression of the phenotypic variation on all putative QTLs plus the residual effects, separating the variance components of each putative QTL and of the residual effects, would be optimum. But detection of multiple QTLs is hampered by two main problems. First, computational work involved is almost infeasible for large numbers of QTLs. Second, many genetics models have to be compared, and thereby, problems of model selection arise.

Zeng (1994) presented a method called « Composite Interval Mapping » which addressed the problem of adequate separation of effects of possible multiple linked QTLs on one same chromosome by combining interval mapping with multiple regression. The method is an interval test in which the test statistic on a marker interval is made to be unaffected by QTLs located outside a defined interval. This is achieved by fitting other genetic markers in the statistical model as a control when performing interval mapping (use of multiple markers). Compared with IM, this method has the advantage of confining the test to one region at a time. This method ignores possible epistasis between loci.

Jansen (1993) presented a similar method called « approximate Multiple-QTL method », or « MQM », which was then developed by Jansen and Stam (1994) and integrated into the software MapQTL5. A first selection of marker cofactors is carried out by multiple regression and backward elimination, so building a first hypothetical multigenic model with a reduced residual variance, and with marker cofactors taking the role of assumed QTLs. Then interval mapping is moved over the successive intervals of the chromosome for the detection of new QTLs. The approximation aspect of the method is that the regression model is based not on the already detected QTLs themselves but on their neighbouring markers. By this way, a single segregating QTL is fitted in a background of genetic cofactors which absorb most of the genetic effects of their nearby QTLs from the residual variance. As a result, the power of the QTL analysis is enhanced. Genetic effects of the separate QTLs, i.e. the single fitted QTL plus the others as represented by cofactors, are modelled as additive fixed effects, not taking into account the possible QTL-by-QTL interactions (epistasis). This method is particularly useful after a QTL with important effect has been mapped on a chromosome, for making sure that the detected QTL does not mask another QTL with smaller effect.

However, it should be noted that, even when it is detected that a specific chromosome contains multiple QTLs, large data sets may still be required to unravel the separate effects of closely linked QTLs. Another aspect is that, if there are missing information in marker genotypes, calculations can become very long. The limitation of the cofactors to a reasonable number (with a maximum of say, 8) may be necessary for allowing the application of the method to many traits.

Jansen (1993) also proposed a strategy for the detection of 0, 1, or multiple QTLs on one same chromosome, based on the use of selected cofactors and on the comparison of models using AIC criterion (Akaike 1974).

1.6.7. LOD significance threshold for QTL detection

For controlling the Type I error, tests in biological research are usually carried out with α (p-value threshold) = 0.05.

When scanning a whole genome for detecting a QTL, the problem arises about the global risk of the operation. The null hypothesis is that there is no QTL over the whole genome. If testing is carried out by IM method every 1 cM on a genome of around 2,000 cM, every run of the software generates more than 2,000 individual tests. Even if there is no QTL for the trait, there is a probability of 5 % to find one for each test, and therefore « false positive » QTLs will be found anytime. For reducing this risk, each test should be carried out with a risk α much lower than 0.05. However the method used by IM for QTL detection is the calculation of a likelihood function and of a LOD score. Thus the new question is how to find a LOD significance threshold equivalent to a global risk α = 0.05 for any run. Larger the genome, and higher the number of tests to be performed. Therefore the threshold depends mainly on the genome size : larger the genome and higher the threshold. A simple LOD score is approximately related to a χ^2 distribution, but because of linkage, the tests on neighbouring positions on the genome are not independent, and only the « peak » LOD scores are considered on a QTL likelihood map (Van Ooijen 1999).

Van Ooijen (1999) proposed four tables issued from stochastic simulation (i.e. Monte Carlo), taking into account four types of experimental populations of diploid species (back-cross with 2 QTL genotypes, recombinant inbred (RI) lines with 2 QTL genotypes, F2 with 3 QTL genotypes, and F1 full-sib family of a cross between non-inbred parents with 4 QTL genotypes). In each table, a series of chromosome map length is considered, from 50 cM to 250 cM. These calculations are based on the hypothesis of the normality of the trait. If the trait exhibits a deviation from normality, the threshold should be higher.

« Permutation tests » (many authors, including Churchill and Doerge 1994) are another method for choosing a LOD significance threshold. It is a resampling method to obtain empirical significance threshold values. A large set of iterations are performed (at least 1000 iterations). In each iteration, the quantitative trait data

are randomly permuted (i.e. sampled without replacement) over the individuals while the genotypic marker data remain fixed. Subsequently, Interval Mapping is done on the thus obtained data set. In these conditions, there is no biological reason for a QTL to be found, except only false positives. The maximum LOD score over all linkage groups is registered in each iteration. After a large set of iterations, a distribution of maximum LOD scores obtained only by chance is recorded. For a Type I error $\alpha = 0.05$, we identify the value of maximum LOD score which is overtaken by less than 5 % of the recorded values of the distribution.

This method of permutation test, included in MapQTL5 for Interval Mapping, avoids the problem of deviation from normality. Another interest is that the threshold is established based on the same data for which the threshold is required. According to Churchill and Doerge (1994), factors that affect the experiment wise error include : the sample size, the genome size, the number of markers (normally stable in one same QTL research project), but also the number of QTLs that influence the trait, and the magnitude of the effects of the QTLs. These two last factors normally change with every trait, which normally makes necessary to re-estimate the LOD significant threshold for every trait. But a practical difficulty is that each series of permutations takes many hours of computation time.

Using a significance threshold avoids mixing real QTLs and false positives due only to random). When large populations are studied, QTLs with weak effects (in some cases less than 1 % of the total phenotypic variation) can be effectively detected. They can be useful for analysing the genetic determinism of traits and the genetic variability of plant response to the environment. However the control of Type I error is a general limitation of power of the QTL-mapping approach. In order to identify a QTL, one has to perform very stringent tests for avoiding false positives. Therefore, small QTLs fall under significance threshold and stay mixed with uncontrolled effects. As a result, only a limited fraction of the genetic variation is explained by the identified QTLs. But the positive aspect is that the most important effects of genetic expression are normally discovered. Considering the aim of Markers-Assisted Selection, QTLs with small effects may often have little interest. In another approach, « genome-wide » selection, or « genomic selection »

omit the significance testing and simply estimate the effects of all chromosomal positions simulaneously (Meuwissen et al. 2001).

Usually, the peak LOD score is the first information provided for a QTL. It provides information about the position and the significance of the QTL. Moreover there is a positive relation between on the one hand the peak LOD score, and on the other hand the percentage of explanation of phenotypic variance by a QTL (%exp) which is the real indicator of the importance of the effect of the QTL. Moreover, a high peak LOD score and a narrow LOD support interval are associated with the precision of the localization of the QTL. Another important aspect for Markers-Assisted Selection is the distance of the closest markers to the QTL most probable position. The value « %exp » of the effect of the QTL can be added to that of the other QTLs for estimating the global percentage of explanation of the phenotypic variation of one trait by the QTLs.

1.6.8. MapQTL5 software for QTL mapping

MapQTL5 (Van Ooijen 2004) is a software for QTL mapping in experimental populations of diploid species (F2 populations, Back-Cross populations, Recombinant Inbred Lines populations, CP populations of outbred species). Analysis is based on the use of three separate data-files (with Xxx as prefix name of the files):

a) Xxx.map : or the “map file. It contains the positions of all the marker loci, in centi-Morgan, on the linkage groups corresponding to the chromosomes of the haploid genome

b) Xxx.loc : or the “locus genotype” file (loc-file). It contains the genotypic data, i.e. the two alleles of every marker and every progeny of the experimental population. For each marker, the “segregation type” indicates the number of different alleles from each parent and the “phases” of those alleles.

c) Xxx.qua : This file holds the “phenotypic” data (or estimations of genetic values) of the quantitative traits of all individuals.

The segregation type-codes for an outbred population are :

- <ab x cd> with the possible genotypes ac, ad, bc, and bd (code -- if there is a lacking data)
- <ef x eg> with the possible genotypes ee, ef, eg, fg
- <hk x hk> with the possible genotypes hh, hk, kk, or hh and k- in case of dominance with ratio 1:3 (for some AFLP markers)
- <lm x ll> with the possible genotypes ll and lm
- <nn x np> with the possible genotypes nn and np

The phase (given for each marker) determines the possible associations between alleles of two loci linked on one same chromosome. For instance with segregation <ab x cd> in an outbred population, the four possible phases for one locus are presented in table 1.

Table 1 : Distribution of the four alleles in the progenies, depending on the four phase patterns.

		Parent A		Parent B	
Segregation	Phase	Chromosome 1	Chromosome 2	Chromosome 1	Chromosome 2
<ab x cd>	(0 0)	a	b	c	d
	(0 1)	a	b	d	c
	(1 0)	b	a	c	d
	(1 1)	b	a	d	c

In an outbred population, any QTL is assumed to segregate according to < ab x cd> pattern, in phase (0 0).

The methods of QTL mapping are :

- Kruskal-Wallis (for non-normally distributed traits)
- Interval Mapping
- Restricted Multiple QTL Mapping (rMQM)
- MQM.

« Kruskal-Wallis » is a non-parametric method, based on ranks, available for trait-data with no normal distribution. The test is performed on each marker-locus independently from all other markers. No use is made of the linkage map. For each

marker position, a statistic K* is calculated (Lehmann 1975). Under the null hypothesis (no segregating QTL), this statistic is distributed approximately as a χ^2 distribution with the degrees of freedom equal to the number of genotype classes minus one. This number of degrees of freedom varies from one marker to the other depending on the type of segregation (4, 3, or 2 possible genotype classes). For obtaining an overall significance level of about 0.05, it is recommended at least a p-value $\alpha = 0.005$ on individual tests by χ^2.

« Interval Mapping » (IM) is available for traits with normally distributed data. A QTL likelihood map is calculated. By use of the « Permutation test », a LOD significance threshold is chosen. The « peak » position with the largest LOD on the linkage group is the estimated (most probable) position of the QTL on the map.

« Automatic Cofactor Selection » (ACS) is aimed at preparing the utilisation of rMQM and MQM methods. It is a multiple regression modelling programme, with backward elimination of non-significant factors, able to determine, among all the markers on the map, or among a subset of markers selected by the user, which ones are worth being kept as cofactors in the model selection. The selected model is the one that maximizes the value of the $^e\log$(likelihood) minus a penalty for the number of free parameters (k) in the model. This is equivalent to the minimization of the AIC criterion. Obviously, adding more cofactors increases the percentage of explanation of the phenotypic variance by the model, but many cofactors may be non significant. The optimal model is « parcimonious » : it combines a large explanation of the variance and a low number of parameters.

$$AIC = -2\,[^e\log(L) - k] \qquad \text{(the lowest is the best)}$$

Each cofactor is equivalent to one QTL with 4 genotypic classes, and therefore it includes 4 regressors in the regression model. Thus it generates (4-1) = 3 parameters in the model. One more parameter is added for the general mean of the phenotypic data. For example, a model with 2 cofactors has 7 parameters ; a model with 5 cofactors has 16 parameters. For using the AIC criterion, the number of parameters should not be too large, preferably less than 2* root(nb of phenotypic observations) (Sakamoto et al. 1986).

« rMQM » and « MQM » implement the interval mapping method on the base of a chosen set of cofactors issued from ACS. In analysing one interval on one chromosome, rMQM takes into account all the cofactors except those located on the analysed chromosome. MQM takes into account all the cofactors except that of a marker flanking the analysed interval. As a consequence, for a chromosome with no cofactor, rMQM is equivalent to MQM.

With rMQM, the H0 model (based on the hypothesis of no QTL) is calculated at the beginning of each chromosome for the whole chromosome. With MQM, the H0 model is first calculated at the beginning of each chromosome, taking into account all the cofactors, and recalculated on the two intervals flanking a cofactor after having temporarily excluded this cofactor. Of course the H1 model (based on the hypothesis of a QTL) is calculated for each interval of the map.

Whereas IM calculations are very fast, and rMQM calculations rather fast, MQM calculations may take a long time, which requires a specific organisation (using two computers or more, using rMQM on linkage groups including no cofactor, using MQM only on linkage groups including cofactors). Even if it is not the optimum statistical approach, it is suggested to first look for QTLs by using IM, so allowing a first selection of cofactors. Then rMQM is performed, and a new selection of cofactors is made. rMQM can be performed once more with the new set of cofactors. Then each linkage group bearing cofactors is analysed individually, followed by a modification of cofactor selection if necessary. Thus a stabilized identification of QTLs with maximum possible LOD scores is achieved. Along the process, LOD scores are generally increased, whereas the %exp is not changed much. This makes possible the identification of new significant QTLs by getting higher levels of the LOD scores, even if the effects of those QTLs are low.

For the selection of cofactors, the markers neighbouring the detected QTLs are tested in the « Automatic Cofactor Selection » procedure. The subset of selected markers is used for building an initial multivariable regression model of the phenotypic variation. This model is tested by estimating the deviance (ML-EM, Maximum Likelihood method and EM algorithm). The residual variance and the

percentage of explanation of the phenotypic variance by the model are estimated. Then the effect of the possible elimination of each cofactor is tested by comparison with the initial model ; a cofactor is eliminated if this elimination does not reduce significantly the likelihood, which means that the residual variance is nor increased significantly and that the percentage of explanation of the phenotypic variance by the model is not reduced significantly. At each step, only one cofactor is eliminated. After elimination of a cofactor, the resulting model built with all the other cofactors is used as the new initial model for testing the reminding cofactors. This iterative process stops when any more cofactor elimination would result in a significant degradation of the regression model. The cofactors finally selected are used for MQM analysis.

In IM, rMQM, or MQM methods, the software always proceeds by the interval mapping method. For one trait, the population variance (usual ML estimate) is calculated and displayed in the « Session Info » tabsheet. In IM, this population variance is used as the basic H0 variance at all the positions of the genome. In rMQM and MQM, a new estimation of the H0 variance, lower than, or equal to, the population variance, is calculated at the beginning of each chromosome, and the difference with the population variance is expressed in % of the population variance (in « Session Info » tabsheet). Then, for each tested position (H1 hypothesis), in the « Results » tabsheet, a new residual variance estimated after fitting the QTL in hypothesis H1 is calculated and displayed, and the difference with the H0 variance, specifically due to this QTL, is expressed in % of the population variance (Table 2).

Table 2 : Informations about variance estimations provided for one trait by MapQTL5 for rMQM or MQM methods.

Line	Type	Variance	Difference	% of Vpop
Session info	Population	0.01054950		
Session info, group n	H0	0.00927523	0.00127427	12.1
Results, group n, position k	H1	0.00796500	0.00131023	12.4

As a synthesis, the percentages of explained variance always refer to the global value of the population variance. In the « Session Info » tabsheet, this percentage indicates the part due to cofactors with the H0 assumption at the initial position of a linkage group. In the « Results » tabsheet, for each position, this percentage indicates the additionnal part of variance due to the difference between H0 assumption (no QTL) and H1 assumption (existence of one QTL) ; the residual variance due to uncontrolled effects (error) is also displayed.

When examining the results from MQM analysis on one group bearing a cofactor, it should be reminded that the results in the two intervals flanking the cofactor are obtained in conditions different from those prevailing in the other intervals (this cofactor is temporary excluded). Thus discontinuities can be clearly observed between the two types of intervals at the levels of the LOD score, of the residual variance, and of the %exp.

1.6.9. Towards Markers-Assisted Selection

The opportunity to select varieties based on the complementary genotypic information brought by molecular markers at very early stage, added to phenotypic measurements, is very attractive to plant breeders. For traits where a low heritability makes phenotypic evaluation costly, molecular genetic information, independant from the environment, would be very useful.

In autogamous plants, MAS can be used to facilitate selection when developing inbred lines by the pedigree method. After QTL identification, the favorable alleles can be selected in an enlarged F2 populations before developing the next generations by selfing. MAS can be used also to introgress favorable alleles by recurrent backcrossing, which is probably the most important application of MAS today. This method can be enlarged to the pyramiding of different genes in one same genotype used as the back-crossed parent. For outbred species with vegetative propagation, selection can be applied to immature individuals, even before they develop the character on which the adults are selected.

Lande and Thompson (1990) presented the general methodology for integrating molecular genetics and conventional selection on phenotypes (MAS), based on

selection indices. MAS was considered for the improvement of a single character by individual selection, and was restricted to only the additive genetic effects of the QTLs. The efficiency of selection could be increased substantially by using MAS after the hybridization of selected cultivars and initial QTL detection. The additive effects can be estimated by multiple regression of individual phenotypic values on marker genotypes. The proportion of the additive genetic variance explained by the QTLs is related with the heritability of the trait (h^2) and the number N of individual genotypes included in the detection study. Consequently, for traits with moderate or low h^2, where MAS should be more wishable, the chances of QTL detection with small sample sizes are low, unless the QTL explains a substantial proportion of the genetic variance. The authors also underline that marker-QTL associations are continually eroded by recombination.

Many papers examined the results from experiments and simulations for determining the real potential of MAS. Hospital et al. (1997) used computer simulations for comparing Marker-Assisted Selection based on an index combining the phenotypic value and the molecular score of individuals, and phenotypic selection alone. Young (1999) presented a review of some of the difficulties of MAS application, including a successful case for soybean cyst nematode resistance. In spite of many favourable aspects, the lines issued from MAS were not uniformly resistant. Accuracy of the estimation of genetic effects is important for MAS success. Frequent weaknesses are due to small populations, a single environment, or just one generation. One main conclusion is that small populations are not adequate for QTL mapping. MAS projects need to utilize better scoring methods, larger population sizes, multiple replications and environments, various genetic backgrounds, and independant verifications.

Although successful MAS for introgression of major genes is plentiful, notably in rice for blast resistance and for bacterial blight resistance (Darlene et al. 1997; Toojinda et al. 1998), MAS based on QTLs is much less documented. Ahmadi et al. (2001) reported about two studies in rice, one for the introgression of alleles for root depth and tolerance to water stress, and the second for the improvement of partial resistance to rice yellow mottle virus. They underline the importance of the initial QTL analysis, depending on various parameters and notably the methods

used for analysing the data. Another limitation can be due to the large confidence interval for QTL location, sometimes up to 30 cM. Epistatic interactions between QTLs may also influence the result of one QTL introgression. A software taking epistasis into account in the framework of composite interval mapping (Wang et al. 1999) would allow further improvement of the precision in QTL analysis.

Due to high costs and long durations of the studies, it has been common practice to estimate QTL effects from the same data used for QTL detection and mapping. With this approach, the QTL effects, which represent the key factor of MAS efficiency, are generally overestimated, due to statistical sampling and GxE interactions. Simulations indicate that the upward bias can be severe for low N and h^2 (Melchinger et al. 1998). Therefore it was suggested to map QTLs with one data set (calibration), and based on this information, to estimate QTL effects with another independent data set (validation). Moreover, stability of the QTLs over the environments must be assessed. As far as the expression of many genes is submitted to the influence of the environment through complex genetic regulation networks, many QTLs can be associated with very specific effects due to the environment and non reproducible in other conditions (QTL x E interactions).

Strauss et al. (1992) expected that the potential of MAS in forest tree breeding would be limited in non-hybrid populations (populations not issued from controlled crossing). But « Marker-aided selection within individually mapped full-sib families can substantially aid phenotypic selection, but only where large restrictions of genetic base are tolerated, trait heritabilities are low, markers are able to explain much of the additive variance, selection intensities within families are high compared with that among families, and very large numbers of progeny are examined ». Actually this scenario is very close to that which one can consider for rubber. The authors continue : « Consideration of trait characteristics suggests that marker-aided selection will be most efficient in direct selection with high-value, low-heritability traits such as height and diameter growth. These traits, however, often show genotype-by-environment interactions and unfavorable genetic correlations with other desirable traits, and are likely to be controlled by a large number of minor genes rather than relatively few major ones. Traits with the most potential for

marker-aided selection in non-hybrid tree populations will therefore be strongly inherited ones for which phenotypic assay is difficult ».

Stability of the QTLs over generations is a concern. Experimental populations in which QTLs were detected, usually stay at linkage desequilibrium (LD) during the first mating generations. LD is necessary for searching favorable alleles among a wide population, and more precisely for MAS selection of genotypes in such a population. This is the case when parental genotypes (genitors) are to be selected from progeny testing (inter-family selection). The situation is different when the candidates to selection are the progenies themselves (intra-family selection). In this case, there is only one generation from the parents to the progenies, and QTL detection does not require LD in the population. From one family to the other, the known QTLs can be re-estimated without any new genetic mapping, by targeting the genome areas where the QTLs were first detected. This situation, much more favorable to MAS application, is met in the case of the rubber tree.

In general, MAS is not a substitute to phenotypic selection but a complementary source of information for improving the accuracy of genetic value estimations. With the development of SNP markers and of associated DNA chips, genomic selection is now developped in France on dairy cattle, with the aim of being partially substituted to the expensive progeny testing. Although genotyping costs follow a decreasing trend, it must be acknowledged that they still are an important limitation to MAS application, notably for the rubber tree.

2. MATERIAL AND METHODS

2.1. Plant material

In the Wickham population of the outbred species *Hevea brasiliensis*, the non-related clones RRIM600 and PB217 were chosen in November 1999. RRIM600 is a quick starter whereas PB217 is a slow starter. Moreover, RRIM600 and PB217 were assumed to be tolerant and susceptible to water stress respectively, although there was no sound evidence of this assumption. The cross RRIM600 x PB217 was performed by hand pollination at the Rrit-Doa Chachoengsao Rubber Research Centre (Crrc) in 2000, and a F1 family of more than 600 progenies was obtained. DNA was extracted from leaves for checking the legitimacy of these trees, and a set of 334 legitimate seedlings was selected for genetic mapping. A subset of 196 progenies was taken at random for field phenotyping. These progenies were multiplied by budding and planted in June 2002. Rootstocks were issued from seeds of RRIM600 female origin.

2.2. Genetic mapping

Genetic mapping was carried out at Cirad (Montpellier, France) on the 334 genotypes by use of SSR markers in 2002 ; it was continued by AFLP markers on the subset of 196 genotypes in 2005 (Prapan et al. 2004). The two parental genetic maps were built by use of the software Mapmaker 3.0 with the back-cross option adapted to the double pseudo-testcross method (Grattapaglia and Sederoff 1994), then a consensus map was established by use of bridge-markers, which allowed the merging of homologous linkage groups, with JoinMap 3.0 software (Van Ooijen and Voorips 2001). This consensus map, further used for QTL detection, was based on 243 SSR and 184 AFLP markers (427 PCR-based markers overall). The expected 18 linkage groups corresponding to the chromosomes of the haploid genome were found for the 3 maps, with total lengths of 1,848 cM, 2,111 cM, and 2,075 cM for RRIM600, PB217 and the consensus map respectively. The average

distances between adjacent markers were of 6.00 cM, 7.23 cM, and 5.07 cM on the three maps respectively. Table 3 presents the structure of the consensus map.

Table 3 : General structure of the consensus genetic linkage map build from the F1 population RRIM600 x PB217. Genetic lengths and number of markers for the 18 linkage groups, average and maximum ranges between adjacent markers.

Linkage group	Length (cM)	Nb markers	Mean range (cM)	Maximum range (cM)
1	89.1	20	4.69	15.49
2	125.9	24	5.47	21.15
3	108.2	24	4.70	15.35
4	109.2	27	4.20	8.95
5	135.2	32	4.36	19.11
6	106.9	19	5.94	32.77
7	105.0	20	5.53	23.91
8	116.3	37	3.23	10.90
9	105.0	19	5.83	15.47
10	173.0	39	4.55	14.86
11	117.3	18	6.90	20.00
12	99.3	24	4.32	15.11
13	122.9	21	6.15	23.47
14	117.5	22	5.60	21.55
15	126.9	23	5.77	18.13
16	118.7	21	5.94	16.24
17	98.8	23	4.49	22.19
18	99.7	14	7.67	20.02
Total	2074.8	427	5.07	32.77

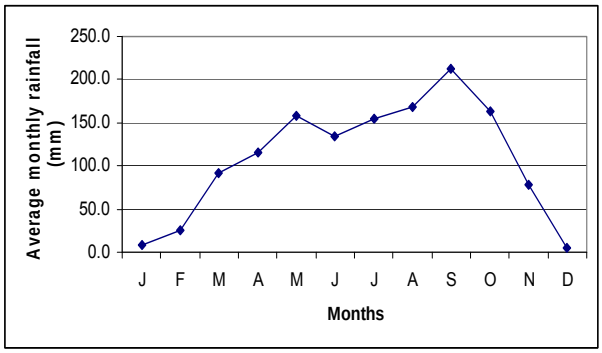
2.3. Ecological conditions of the field experimentation

The field trial was planted at Crrc, a suboptimal site for rubber cropping due to limited rainfall (table 4), uneven rainfall distribution, long annual dry seasons (between November and April), and sandy fast-drying soil. Moreover, the field was affected by a lateritic hard-pan lying at 0.5-0.8 m below ground level, reducing taproot development and access to water in the soil. Although the statistics of the last 20 years indicate, for an average dry season, 3 months with rainfall below 50 mm (figure 6), years with dry seasons including 4 dry months are frequent.

Table 4 : Monthly rainfall at Rrit-Doa Chachoengsao Rubber Research Center.

Months	Moy 1989-09	2001	2002	2003	2004	2005	2006	2007	2008	2009
1	8	1	1	0	16	0	0	0	6	0
2	26	11	43	48	28	0	31	24	44	0
3	92	135	54	230	91	62	101	155	69	166
4	116	104	144	70	100	120	86	248	235	130
5	159	228	215	150	92	117	221	260	147	185
6	135	206	131	189	180	92	138	115	220	107
7	154	103	138	160	124	108	194	196	282	106
8	169	135	230	105	166	149	195	59	222	118
9	213	166	223	250	183	351	404	206	382	191
10	163	129	88	86	82	111	164	77	249	135
11	77	6	35	0	0	117	9	23	31	40
12	5	21	15	0	0	18	0	0	1	1
Total	1317	1244	1315	1288	1062	1245	1542	1362	1886	1177

Figure 6 : Average monthly rainfall over 21 years, from 1989 to 2009.



The average minimum monthly temperature over the period 2001-2009 at Crrc was of 23 °C, and it varied from 17.2 °C to 25.6 °C. The months with the lowest minimum temperatures were from November to February. The average maximum monthly temperature was of 33 °C, and it varied from 30 to 37 °C (months of February, March and April). Yearly insolation varied from 2176 to 2483 sunny hours during the period from 2001 to 2009, which was able to generate high transpiration levels and to induce frequent water stress.

2.4. Experimental design of the field trial

A flat field of 6.32 ha was planted with a planting density of 625 trees per hectare (4 x 4 m), slightly higher than the usual density in rubber cropping (500 t/ha). For each progeny there were 16 budded trees distributed in 4 plots with 4 trees per plot. For controlling the variations of the environment between plots, an α (0, 1)-design (Patterson and Williams 1976) was set with 4 full replications, and 25 incomplete blocks of 8 plots per replication (4 x 25 x 8 = 800 plots). Every full replication included all the 196 progenies in 196 plots (+ 2 plots for each parent), i.e. 200 levels of treatment. Every block included 8 genotypes (progenies or parents). Randomisation of the design was carried out with a Cirad software (J.P. Jacquemoud, unpublished.) so that every progeny (4 plots in 4 different blocks) might be compared with 4 x 7 = 28 other progenies or parents) in similar conditions. As a result, among the 19,900 possible pairs of varieties, 17,100 were in no block, and 2,800 were in one block. A total of 3200 trees contributed to the experiment.

3200 trees = 4 trees/plot x 8 plots x 25 incomplete subblocks x 4 full-replications

An extra number of 500 trees were planted besides the trial for the measurement of biomass increase along time by felling 25 trees every year.

The genotypes were coded from n°1 to n°196. Four other codes were given to RRIM600 (n°197, n°198) and PB217 (n°199, n°200). The parents, although not required for QTL mapping, were planted in the trial as controls for comparison with their 196 progenies. Along the duration of the study, many results indicated that the genotype planted in the trial as PB217 might not be really this clone. For those reasons, results for PB217 were not presented. A small test realized on 20 trees of the trial, among the progenies, showed that one of them was not true-to-type.

2.5. Data management

The Genmap data were recorded and stored on a tabsheet with one line per tree (3200 trees). In the columns, there were eight variables for the characterization of the trial :

- Ntree (tree number, from 1 to 3200)
- Line (line number, from 1 to 76)
- Row (row number, from 1 to 48)
- Codec (genotype number, from 1 to 200)
- Plot (plot number, from 1 to 800)
- Rep (replication number, from 1 to 4)
- Share (share number, from 1 to 8) = specific blocks for latex diagnostic data, over 793 trees.
- Block (subblock number, from 101 to 425).

The other colums were devoted to measurements and observations, as well as to the status of each tree at periodic dates (living or dead, still included in the trial or not, etc.). The names of the columns, V1, V2, V3, …, were created in the chronological order of data recording, and a Word file (VariableNames-Genmap.doc) was used for storing the definitions and characteristics of these variables.

Two other files were created for recording biomass data on the 175 trees located outside of the trial, and MMD_0 data on 374 trees in the trial.

2.6. Heritabilities, BLUP estimations and correlations

The distributions of the data were examined by use of « Sas insight » software. Whenever necessary, a transformation was applied to the data for normalizing them. The log transformation was used for the 14 production traits : Y = log(X). The root transformation was used for sucrose content : R = square root (X).

A mixed model was used for analysing the data of individual trees. The objective was to estimate the heritabilities of the traits and the genetic values of the genotypes (BLUPs, Henderson 1975).

The model was :

$$Y_{ijkl} = \mu + r_i + b(r)_{ij} + G_k + Gxb(r)_{ijk} + \varepsilon_{ijk}$$

Fixed effects :

- r_i indicates the full-replications (from 1 to 4)
- $b(r)_{ij}$ indicates the subblocks in each replication (from 101 to 425)

Random effects :

- G_k indicates the genotypes (from 1 to 200)
- $Gxb(r)_{ijk}$ indicates the interaction between the genotypes and the blocks
- ε_{ijk} indicates the residual effect.

The procedure « proc mixed » of SAS statistical software (2003) was used. Broad sense heritabilities were estimated at individual tree level :

$$h^2l = Var\ (G) / (Sum\ of\ the\ variances\ of\ the\ three\ random\ effects)$$

Only the measured data were sumbmitted to the calculations of BLUP estimations. Traits calculated from other measured traits (such as girth increments or heigh increments, ratios, means of several traits, etc.) were calculated from the BLUP estimations of the measured traits.

Coefficients of correlations between couples of traits were estimated at the level of the BLUP estimations of the n = 196 genotypes (degrees of freedom df = n-2 = 194 ; significance threshold r = 0.14 for α = 0.05). As far as the BLUPs are estimates of genetic values, these correlations are very similar to genetic correlations. These coefficients were calculated by use of Xlstat softwares.

A multiple regression analysis was used for explaining each of the 14 production traits by use of one same set of 6 early selection criteria: G59 (girth just before tapping), Gi5964 (girth during tapping in 2007), Drc07 (average dry rubber content measured in 2007 from LD analyses), Suc07 (average sucrose content measured in 2007), Pi07 (inorganic phosphorus content measured in 2007), and Rsh07 (thiol content measured in 2007).

The dependence of production traits on the girth of the trunk was taken into account for a second analysis of girth-adjusted production traits. To do that, the BLUPs of the production traits were estimated by putting the girth G59 (measured just before the first tapping) as one more fixed effect in the statistical mixed model. Thereby, G59 played the role of a cofactor, and new heritabilities and BLUP estimations were calculated for the girth-adjusted production traits.

Concerning heritabilities, the statistical models were not exactly the same for analysing the data of : a) growth and production traits, b) latex diagnostic traits, and c) molar mass distribution traits. Therefore, the comparison of heritabilities among these three types of traits would be not rigourously exact. By contrast, heritability comparisons within each type of trait is legitimate.

2.7. Traits measurements

The three main categories of phenotypic traits were : growth, rubber production and related traits (latex diagnostic, plugging index), and the molar mass distribution traits of the rubber chains (MMD_0). Measurements and scorings were carried out individually on each tree (there were generally more than 2400 measurable trees). Plugging index and latex diagnostic series were measured on a sample of 793 trees. MMD_0 traits were measured on latex films issued from 374 trees.

Other traits were based on counting, scoring, or measurements :

- number of living trees at different ages
- defoliation earliness

- die-back index
- leaves dimensions
- bark depth
- fresh biomass components of the trees (from 175 trees).

Many traits were mesured repeatedly at different ages. The dates of measurement were indicated in months after planting in the labels of the traits.

2.7.1. Growth

Growth began before planting, with the initiation of scion-sprouting after rootstock cutting-back in nursery (May 2002), and the budded plants in bags had their first growth-unit already developped on the planting day. Therefore the first girth at 10 cm-high (Ga1), and the first height (H1) measured in month 1 must be considered as resulting from the first period of increment since May 2002.

From month 1 to 23, due to the small height of the plants, the girth of the trunk was measured at 10 cm-high (Ga). The measured traits were : Ga1, Ga3, Ga6, Ga12, Ga18, and Ga23. From month 18 to 89, girth was measured at 1.0 m-high and 1.7 m-high, and the average of the two measurements was calculated. The following traits were thus obtained : G18, G23, G31, G36, G43, G47, G53, G59 (before tapping), and G64, G71, G76, G79, G82, G85, and G89 (during tapping).

The corresponding height traits were : H1, H3, H6, H12, H17, H23, H30, H36, H43, H47, H53 (before tapping), and H67and H79 (during tapping). After 53 months, the canopy had closed and the trees had become higher than 8 m-high. It was thus difficult to measure height. Therefore the two last height measurements H67 and H79 were carried out in January 2008 and January 2009 during defoliation.

The measurement dates of growth before tapping were chosen for taking into account the alternance of the rainy (R) and dry (D) seasons R1, D1, R2, D2, R3, D3, R4, D4, R5, and D5. Thus the following increments were calculated : Gai1 and Hi1 (season R1a), Gai13 and Hi13 (R1b), Gai36 and Hi36 (R1c), Gai612 and Hi612 (D1), Gai1218 and Hi1217 (R2), Gai1823, Gi1823, and Hi1823 (D2), Gi2331

and Hi2330 (R3), Gi3136 and Hi3036 (D3), Gi3643 and Hi3643 (R4), Gi4347 and Hi4347 (D4), Gi4753 and Hi4753 (R5), and Gi5359 (D5).

Three annual periods of tapping were carried out from month 60 to 64 (2007), from month 72 to 75 (2008), and from month 83 to 88 (2009). Therefore there were girth increments during tapping and rainy periods : Gi5964 (R6), Gi7176 (R7), Gi8285 (R8a), and Gi8589 (R8b), and increments during dry seasons without tapping : Gi6471 (D6), Gi7679 (D7a), and Gi7982 (D7b).

Table 5 provides the names of all the growth traits. From month 1 to 23, diameters were measured with a calliper, and converted into girth. Afterwards, girths were measured with a tape measure. Heights were measured with a graduated telescopic road (equipment dedicated to dendrometry in forest research).

Table 5 : Names of the growth traits, with the corresponding months and seasons of measurement. Ga = girth measured at 10 cm-high ; G = average of girths measured at 1.0 and 1.7 m-high.

Season	Month	Girth	Height	Girth increment	Height increment
R1a	1	Ga1	H1		
R1b	3	Ga3	H3	Gai13	Hi13
R1c	6	Ga6	H6	Gai36	Hi36
D1	12	Ga12	H12	Gai612	Hi612
R1	17/18	Ga18, G18	H17	Gai1218	Hi1217
D2	23	Ga23, G23	H23	Gai1823, Gi1823	Hi1723
R3	30/31	G31	H30	Gi2331	Hi2330
D3	36	G36	H36	Gi2336	Hi3036
R4	43	G43	H43	Gi3643	Hi3643
D4	47	G47	H47	Gi4347	Hi4347
R5	53	G53	H53	Gi4753	Hi4753
D5	59	G59		Gi5359	
R6	64	G64	H67	Gi5964	Hi5367
D6	71	G71		Gi6471	
R7	76	G76		Gi7176	
D7a	79	G79	H79	Gi7679	Hi6779
D7b	82	G82		Gi7982	
R8a	85	G85		Gi8285	
R8b	89	G89		Gi8589	

2.7.2. Biomass

In one part of land neighbouring the trial, 500 trees were kept untapped for the study of fresh biomass evolution. From year 1 to year 7, a total number of 175 trees (25 trees per year) were randomly chosen, extracted and cut into pieces for estimating the importance of the different components of the biomass.

On these trees, the following measurements were carried out :

- G1 : Girth measured at 1-m high (from 2004 to 2009)
- H : Height
- Wtr : Fresh weight of taproot
- Wlr : Fresh weight of lateral roots
- Wt : Fresh weight of the trunk (axis 1 of the trunk until terminal bud)
- Wb2 : Fresh weight of the branches of second order
- Wbo : Fresh weigh of all the other branches
- Nf : Number of fruits
- Wf : Fresh weight of fruits
- Wlea : Fresh weight of leaves

Then, seven equations of prediction (one per year) of the total biomass depending on other growth traits were built by multiple regression for evaluating the biomass acquisition of the trees in the trial. A global equation was built for predicting biomass acquisition whatever the age.

2.7.3. Rubber production

The girth preceding the initial tapping was measured in May 2007 (G59), prior to the initial tapping which occured on June 2. All the trees with a girth higher than 25 cm were opened, and tapping was carried out from June to October 2007, from June to September 2008, and from May to October 2009.

The cumulated production was weighed for 14 successive periods, the duration of which varying from 20 to 45 days. There were five production traits in 2007 (with no tapping interruption during the year, from P71 to P75), three production traits in 2008 (from P81 to P83), and six production traits in 2009 (from P91 to P96).

A tapping schedule was established and never changed, even in case of tapping cancellation by rain. Due to such tapping cancellations, tapping frequency was sometimes not regular, especially in 2008 where annual rainfall was as high as of 1886 mm ; for this reason, the production data were converted in mg/tree/day.

During the first year (2007), a S/2 d/3 6d/7 low-intensive tapping system with no ethephon stimulation was applied to the trial (half-spiral with one tapping every three days). In 2008, the same system was applied, with one ethephon stimulation at the beginning of the period corresponding to P82.

In 2009, an intensive tapping system was applied, combining an increased tapping frequency (d/2) and three stimulations before P93, P94, and P96. Each stimulation (ET 2.5 % 1/1) was equivalent to 25 mg of ethephon per tree per stimulation). Due to misunderstanding, a first stimulation with a low concentration (5 mg/tree) was applied at the initiation of tapping (before P91).

For each tree, two days after each tapping, the cuplump was collected from the cup and stringed on an iron wire attached to the tree. For each wire, there was a metal tag of identification of the tree (line and row numbers). At the end of each period, the wires were collected and transfered to a weighing area, where all the cuplumps of each tree were immediately weighed and the result recorded. Rubber production was weighed with an electronic balance (in centigrams) for each tree, in the form of the cumulated coagulated cuplumps of each period. By this way, there was a variation in cuplump drying, from the cuplump issued from the first tapping to the cuplump issued from the last tapping. However this situation was equivalent from one tree to the other.

Weighing totally dry rubber would have generated important difficulties of management on the drying area, with a considerable reduction in data collection. As far as genetic variability is important, there is a high positive correlation between coagulated cuplumps and dry rubber among the genotypes. This was checked by studying the kinetics of cuplump air-drying over a period of one month, on a sample of 200 cuplumps issued from 200 trees and 65 genotypes tapped the same day (this study is presented in the « Results » chapter).

2.7.4. Plugging index

Normally, plugging index is issued from the measurement of volumes of latex. In our case, the process was adapted by weighing the latex flow during the first five minutes (W1 trait), and the second part of latex flow until cessation due to the coagulation on the tapping cut (W2). Then plugging index (PI) was calculated.

A sample of 793 trees was designed, with one tree per genotype for each of the four replications. The organisation of the work was as following :

- 793 empty bottles with caps and tags (one per tree) were weighed (wa), and then put opened in the tapping cups before tapping
- two chronometers were used in a coordinated way for giving regular signals to the tapper and to the bottle collector
- one signal was given to the tapper every 30 seconds for tapping one tree
- starting five minutes later, one signal was given to the bottle collector every 30 seconds for collecting the bottle, closing it with the cap, and then letting the latex flow in the cup
- the bottles were taken to the laboratory and weighed (wb)
- then the bottles were taken back to the field at the end of the latex flow, and the latex of each tree was poured from the tapping cup to the corresponding bottle
- the bottles were taken to the laboratory and weighed (wc)

Calculations were as following :

- W1 = wb – wa
- W2 = wc – wb
- PI = W1 / (W1 + W2) = (wb – wa) / (wc – wa)

There were three series of measurements of the plugging index (two in 2007, and one in 2009), corresponding to the periods of latex production measurements P73, P74 (low-intensive period), and P96 (end of the intensive period). The traits W1 (production of the first five minutes after tapping) and W2 (production after the first five minutes) were normalized by a log-transformation. Estimations of the genetic values of these traits were made (BLUPs) and used for the calculation of the

plugging indexes of each genotype for each series. The plugging index traits PI73 and PI74 were also normalized by a log-transformation, but it was not necessary for PI96 (normal distribution of natural data).

Drc was measured only for the two series of 2007 : the latex coagulated in the bottles was taken out of the bottles and put to air-drying for one month and weighed (wd). Calculation was as following :

$$Drc = wd / (W1 + W2) * 100 = wd / (wc – wa) * 100$$

The names of the measured traits are presented in table 6.

Table 6 : Plugging index. Names and definition of the measured traits. LW = log-transformed weight.

Year	Series	Type	Trait
2007	1 (P73)	W1	LW173
		W2	LW273
		Drc	DrcPI73
	2 (P74)	W1	LW174
		W2	LW274
		Drc	DrcPI74
2009	3 (P96)	W1	LW196
		W2	LW296

2.7.5. Latex diagnostic

Samples of latex were collected from individual trees and analysed. Rubber was coagulated by acid, and three biochemical measurements were made on C-serum by use of a spectrophotometer : sucrose content (Suc), inorganic phosphorus content, and thiols content (Jacob et al. 1987). The fourth trait, Drc, was measured by drying the coagulated rubber during 24 hours in owen at 70 °C and weighing it; the dry weight was related with the weight of fresh latex (Flw).

A spectrophotometer Shimadzu UV-VIS (ultra-violet and visible light) UV-160 (P/N 204-04550), equiped with a monochromator, a screen (CRT), a keyboard, and a printer was used. It was a double-beam spectrophotometer. In the sample compartment, there was a « reference cell holder » containing the blank used for a

whole series of measurements, and a « sample cell holder » for the successive samples of the series. For each measurement, the optical density of the sample was compared with that of the blank. The measuring wavelength range was 200-1100 nm (as a recall, visible light wavelength spreads from 380 to 780 nm). For LD (latex diagnostic), the following wavelengths were used : a) 627 nm for Sucrose, b) 410 nm for Pi, and c) 412 nm for Rsh. Samples were loaded manually and measured one after the other.

One tube was prepared for each tree with a known amount of water, and latex drops (equivalent to around 50 cg) were collected from the tree into the water. In the protocol, a collection of 7 drops is advised. But in Genmap, it appeared that 12 drops were closer to 50 cg and more adapted.

- The day before latex collection, the empty tube was weighed (« we ») in the lab
- 3.5 ml of a water solution (in fact a 0.01 % EDTA solution) was added with a pipette, and the tube was weighed again (« wo »).
- After collection of the latex and return to the lab, the tube was weighed again (« wl »)
- Then 0.5 ml of TCA 20 % (acid for rubber coagulation) was added, and the tube was weighed again (« wlt »)
- Then the coagulated rubber was collected and put to the owen for drying.
- The serum was then filtered with filter paper.
- From the four weighings : we, wo, wl, and wlt, three differences were calculated : w1 (water solution) = wo-we, Flw (fresh latex weight) = wl-wo, and w2 = wlt-wl (weight of acid solution).
- A dilution factor was calculated : d = (w1+Flw+w2)/Flw.
- Serum was collected from the samples for three separate biochemical reactions (Suc, Pi, and Rsh respectively) before analysis by the spectrophotometer.

Weighing of we, wo, wl, wlt was done with a balance accurate at the level of 1 cg. For the biochemical measurements of Suc content, Pi content, and Rsh content in mM (millimole/liter), the spectrophotomer output was optical density (OD). The conversion of OD into mM (millimole per liter of fresh latex) was made through the

slope K of a linear standard curve which was established for each trait every day of analysis, just preceding the series of measurements. This standard curve was based on standard solutions equivalent to a range of known concentrations. The conversion into mM takes into account the dilution factor.

$$[\text{Content}] = d \times K \times OD$$

Sucrose (Suc) : Anthrone reaction. In concentrated acidic solution, hexoses dehydrate and give a furfural derivative which reacts with anthrone. Fructose reacts quickly even if it is a part of sucrose molecule. Glucose must be warmed to react (a double-boiler was used). By heating, all hexoses (and therefore all sucrose) are measured. Measurement by absorbtion at the wavelength of 627 nm (nanometer). Ref : Ashwell 1957.

Inorganic phosphorus (Pi) : Molybdate-Vanadate reaction. Inorganic phosphorus binds to molybdate and vanadate to form a complex that absorbs at 410 nM. Ref : Tausski and Shorr 1953.

Reduced thiols (Rsh) : Ellman reaction. Reduced thiols react with dithiobis nitrobenzoic acid (DTNB) to form TNB which strongly absorbs at 412 nm. Ref : Boyne and Ellman 1972.

Nine series of latex diagnostic LD72a, LD72b, LD74, LD81, LD82, LD92, LD93, LD95, and LD96 were measured during the periods corresponding to the rubber production traits P72 (two successive latex diagnostics LD72a and LD72b), P74, P81, P82, P92, P93, P95, and P96. Measurements were carried out on individual trees on the sample of 793 trees also used for plugging index measurements. Therefore there were four trees measured for each genotype.

BLUP estimations of Drc, Suc, Pi, and Rsh were carried out for each LD series. As a maximum reasonable number of 120 samples could be analysed per day for LD, each replication of the field trial was split in two parts which were named « shares ». Thus there were 8 shares with around 100 trees per share. The number of subblocks per share was 12 or 13 (there were 25 subblocks in each

replication). One share was analysed per day. A complete LD series was analysed in a duration of 8 days. Due to this organisation, the statistical model used for estimating the BLUPs was taking into account the shares (as blocks) and the subblocks nested in each share, as two fixed effects.

2.7.6. Defoliation earliness

Defoliation earliness was scored from S1 (not yet defoliated) to S5 (completely refoliated), at the beginning of each year in 2005 (Def31, month 31 = January 2005), 2006 (Def43), 2007 (Def55), 2008 (Def66), and 2009 (Def79). In 2007, there were two weekly scorings. In 2008 and 2009, there were 5 and 4 weekly scorings respectively. For each date of observation, an average score was calculated for each genotype. No BLUP estimation was performed for these traits ; the mean scores of the genotypes were submitted to QTL detection.

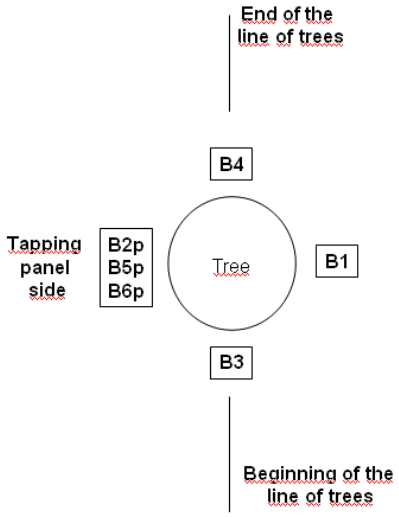
2.7.7. Leaf dimensions

Two series of measurements were carried out in 2006 at 48 months and 52 months. For each series, one leaf per tree was collected on 2308 trees. Measurements were carried out for the fresh weight (FW1, FW2), the length (L1, L2) and the width (W1, W2) of each leaflet. The total leaf area of the three leaflets (LA2) was measured for the second series. The means of the three leaflets were calculated for fresh weight (FW1, FW2), length (L1, L2), and width (W1, W2).

2.7.8. Bark thickness

Bark thickness was first measured in April 2008 on the four sides of each tree (B1, B2p, B3, and B4. Then two repeated measurements were made on the tapping panel side in June 2009 (B5p and B6p).

Figure 7 : Positions of bark thickness measurements.



2.7.9. Die-back

In month 36, a severe die-back was observed, starting from the apical meristems and descending down the branches and the trunk, letting bark and wood tissues dead behind it. Part of the affected trees died, and other trees were severely damaged.

The die-back phenomenon was scored from 1 (dead tree) to 5 (healthy tree with no visible symptom). The genetic influence on this phenomenon was tested by a χ^2 test on the contingency table built from the numbers of trees per score and per genotype. Coefficients and the numbers of trees per score were used for calculting a weighed mean per genotype (die-back index). The die-back index could vary from 0.000 (resistant genotype) to 1.000 (highly susceptible genotype).

Table 7 : Die-back symptoms. Scoring from 1 to 5.

Score	Coefficient	Symptoms
1	1.00	Dead
2	0.75	Not completely dead
3	0.50	Dead branches
4	0.20	Yellow leaves
5	0.00	Healthy

2.7.10. Latex films and rubber macromolecular structure

Molar Mass Distributions (MMD_0) were analysed by Steric Exclusion Chromatography (SEC). For analysing MMD_0 without changing the properties of native rubber, it was necessary to dry the rubber without heating it, and to conserve the samples protected from light and air before analysis. For achieving this goal, latex films were prepared from fresh latex. For preventing the formation of a high percentage of gel, Hydroxilamine sulfate (HAS) was mixed with the fresh latex prior to the preparation of the films.

Preparation of the latex films

Window glass of dimensions 60 x 40 cm were cleaned and prepared : each glass was divided into 12 squares of dimensions 15 x 15 cm. The divisions and identification of each square were marked on the bottom of the glass for avoiding any contact of the latex with the ink of the marker. Empty bottles with caps and tags were weighed (one bottle per tree). After the collection of latex (from 10 to 20 grams from each tree), the bottles were weighed again for determining the weight of fresh latex. The equivalent dry rubber weight was calculated by use of the coefficient 0.40. Then a volume of 0.15 µl of HAS per cg of dry rubber (or 0.06 µl per cg of fresh latex) was added to each latex sample and mixed gently in order to avoid coagulation. Then a small amount of latex was spreaded on each square of the window glasses for making thin layers of latex, easy to dry. Drying was carried out with portable fans, in a air-conditionned room, until the latex films have become completely translucent. The glasses were then put into a box hermetic to air and light. The glasses was piled up, with four coins put at the four corners between two glasses for maintaining a space and avoiding contact between two successive glasses. Silicagel was added into the box, and some closed and empty bottles were added into the box for reducing the amount of air in the box. Then the box was closed hermetically with sticky tape until the date of transportation to the laboratory in Montpellier. On the date of departure, the box was opened in a air-conditioned room, the glasses put on tables, and each film was rolled by hand with a finger and put into a hermetic plastic bag with a tag for identification.

Experimental design

Among the set of 793 trees used for plugging index and latex diagnostic, a subset of 379 trees was taken in replications 1 and 4 (one tree per genotype and replication). Due to problems of coagulation during the preparation, only 374 latex films were prepared for a total of 199 genotypes (genotype n° 135 lacking). From each latex film (issued from each tree), 3 independent samples were cut and taken for analysis (3 laboratory replications), thus taking into account the possible variation between different analyses in one same film.

The experimental design was a nested design with the following levels :

- Two full replications (corresponding to the 2 field replications)
- In each full replication : 3 groups of 6 series of SEC analysis corresponding to the 3 laboratory replicated analyses on each film
- Each series of SEC analysis was composed of 32 film samples.

The 36 series of SEC analysis were coded from 1 to 36 in chronological order of analysis, with 6 groups (1-6, 7-12, 13-18, 19-24, 25-30, and 31-36). Due to lacking genotypes in each full replication, the random allocation of samples into the SEC series was made in two times, separately on each full replication, by use of the Cirad software « α-design », with the following parameters : 192 genotypes, 3 replications (groups), 6 blocks (SEC series) per replication, and 32 plots per block (or series). Therefore, a number of 32 x 36 = 1152 analyses was planned, but in fact, a total of 1057 analyses were performed with success. For statistical analysis, a mixed model was used with the following effects :

- fixed effect due to the 2 full replications (field replications)
- fixed effect due to the 36 SEC series of analyses nested within the full replications
- genetic random effect : 199 genotypes
- random error effect.

For each analysis, the following traits were measured : Gel, M_n, M_w, M_z, M_{z+1}, S1 (area proportional to the number of long chains), and S2 (area proportional to the

number of short chains). The limit between the two areas was chosen visually on the screen of the chromatographer and marked for enabling the computerized calculation of both areas. Polydispersity $I_p = M_w/M_n$, and the ratio of short chains R21 = S2 / (S1 + S2) were calculated. BLUPs and heritabilities were calculated for the traits Gel, M_n, M_w, M_z, M_{z+1}, I_p, and R21.

2.8. QTL detection

MapQTL5 software (Van Ooijen 2004) was used for QTL detection. For traits with normal distributions, the analyses were carried out first by the « Interval Mapping » method, and then by the MQM method. The LOD score threshold was estimated for many different traits by permutation test. In all cases, this threshold was found included in the interval [4.4 ; 4.6]. Therefore one same LOD significance threshold, « LOD threshold = 4.5 », was used for all the traits. For the the die-back index (non-normal distribution), the method of Kruskal-Wallis was used.

The following results were recorded :

- the most probable position of the QTL
- the peak LOD score of the QTL
- the percentage of explanation of the phenotypic variance by the QTL
- the average values of the four phenotypic classes of the QTL (« ac », « ad », « bc », and « bd »).

For making the reading of the results easier, the average values of the four phenotypic classes were transformed into indexes relative to the value of the « ac » class. In this way, the index of the class « ac » was always equal to 100. The index of the class « ad » indicated the effect of the substitution of the allele « c » by « d ». The index of the class « bc » indicated the effect of the substitution of the allele « a » by « b ». And the index of the class « bd » indicated the effect of the substitution of both alleles « a » and « c » by « b » and « d ».

3. RESULTS

3.1. Yearly rainfall and evolution of the living stand

3.1.1. Rainfall

From 2002 to 2009, annual rainfall varied from1062 mm (in 2004) to 1886 mm (in 2008). There was a period of drought from November 2003 to August 2005 (figure 8), including the seasons D2 (dry), R3 (rainy), and D3 (dry). During D3, there were four successive months without any rain (month 30 on figure 9). The mobile mean, calculated for each month with the mean of the 12 preceding months, reached a minimum in August 2005 (month 38), in the middle of the rainy season R4.

Figure 8 : Semestrial rainfall (mm) from May 2002 to October 2009.

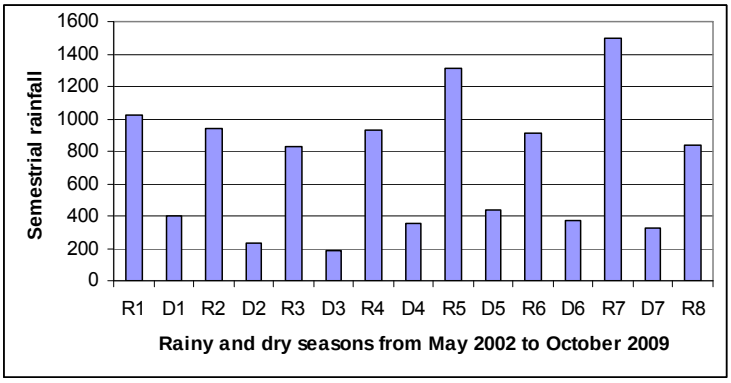
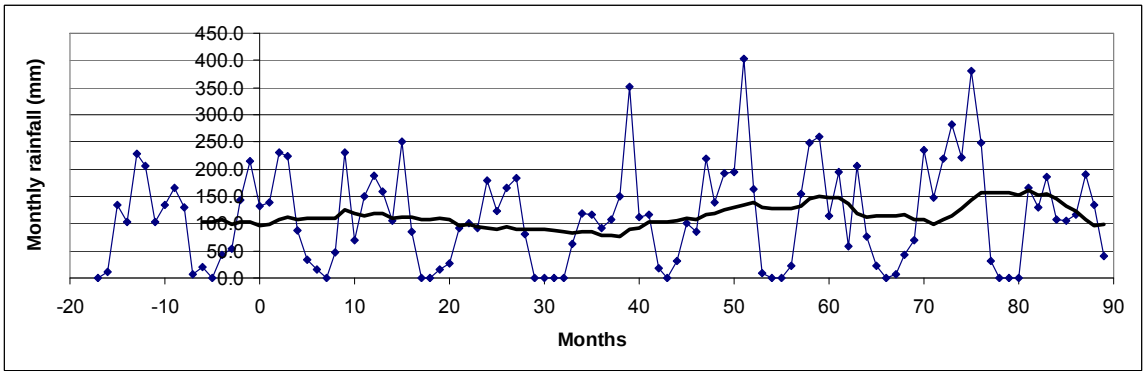


Figure 9 : Monthly rainfall from January 2001 to November 2009. The mobile mean was calculated based on the 12 preceding months.

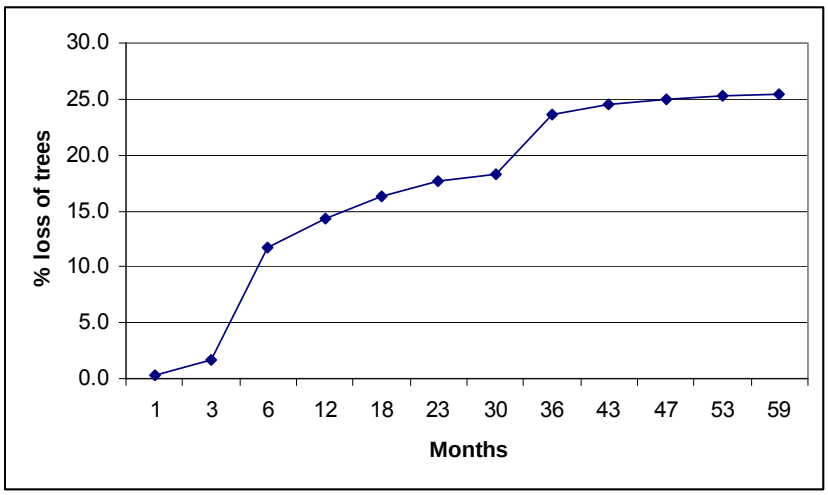


3.1.2. Evolution of the stand

Figure 10 shows that the most important losses of trees occured during the rainy season R1 of the first six months following the planting (12 %), and during the third dry season (D3), from month 30 to 36 (5 %). During the season R1, although rainfall was of 140 mm per month, the young plants had to cope with the planting stress and with the onset of the first dry season.

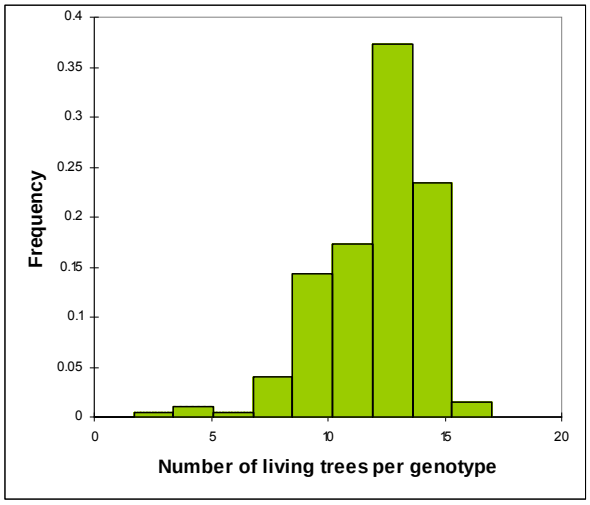
The season D3 was very dry (60 mm per month) and it followed the very dry season D2 (47 mm per month) and the rainy season R3 with limited rainfall (103 mm per month). The phenomenon of severe die-back occured during the season D3. The cumulated loss of trees from month 18 to 43 was of 262 trees, which represented 8 % of the initial stand. At five years of age, the total percentage of lost trees was of 813 trees (25 %).

Figure 10 : Evolution of the percentage of trees lost from planting to five years of age (initial situation = 100 % = 3200 trees planted in June 2002).



At five years, the number of living trees per genotype varied from 3 to 16, but only the five genotypes n° 3, 43, 127, 181, and 195 had less than 8 living trees (figure 11). At each age, a χ^2 test ($\alpha = 0.05$) showed that the number of living trees per genotype was not randomly distributed, thus indicating some genetic effect on the loss of trees, probably related with susceptibility to transplanting and water stress.

Figure 11 : Left-skewed distribution of the number of living trees per genotype at the age of 59 months (nearly 5 years).



Among the 2387 trees living in June 2007 (beginning of tapping) to November 2009, 41 trees were uprooted by a windstorm (linked with Nargis typhoon) in April 2008 ; four trees were suddenly killed, probably by a lightning, and 17 other trees died for unknown reasons. Among the tapped trees, 133 trees developed TPD symptoms, including 29 « brown bast » trees, and 114 « dry cut » trees without any visible bark necrosis. A χ^2 test ($\alpha = 0.05$) showed that these TPD trees were not randomly distributed among the genotypes, thus indicating a genetic effect.

3.2. Study of heritabilities, genetic values, and correlations

Prior to QTL detection, the data were analysed for BLUP estimations of the genetic values of the 196 genotypes, estimation of the heritabilities of the traits and of the correlations between pairs of traits.

3.2.1. Growth

These results cover the first period of growth before tapping, from planting in month 0 (June 2002) to month 59 (May 2007), and the second period from month 59 to 89 (November 2009) which included three periods of tapping of 5 months in

2007, 4 months in 2008, and 6 months in 2009. Biomass evolution was studied from 2002 to 2009 on a specific set of untapped trees.

3.2.1.1. Evolution of growth

Growth in girth before tapping (measured at 1 meter-high) appeared to be close to linear with time, with the regression equation y = 0.54 x - 0.53 (R^2 = 0.98, df = 6). Normally, a rubber plot is « opened » (beginning of tapping) when half of the trees have reached a girth of 50 cm, which is equivalent to a mean girth of 48 cm for all the trees. With this regression equation, it was found that opening could be set at 7 years and 4 months. In the most favorable conditions, rubber trees planted in bags can be opened after 5 years and 6 months, but more often after 6 years. The longer period observed here was due to the limited rainfall of the site (1337 mm per year during the 5 years of growth of the trial), the fast-drying soil with a lateritic hardpan, the occurrence of a severe drought, and the planting density of this trial (625 trees/ha), a little higher than normal.

Table 8 presents the data issued from BLUP estimations of growth in height of the genotypes. For the height increments Hi3036 and Hi3643, negative individual increments were found, which is due to the low increments of these periods and the limited precision of height measurements (imprecision of more or less 50 cm). This explains the high level of variation of Hi3643. The very low height increment of this rainy period was striking. It was probably due to the severe stress suffered by the trees during the preceding drought period, with an impact on the terminal buds.

Table 9 presents the data issued from BLUP estimations of growth in girth of the genotypes. The coefficients of variation of H53 and G59 were of 4 % and 3 % respectively. The progeny with the highest girth G59 was 9 % higher than the mean, whereas the progeny with the highest height H53 was 10 % higher than the mean. Thus the variability between the genotypes for growth traits was rather low.

The average lengths of the first four growth-units GU1, GU2, GU3, and GU4 were of 30.2 cm, 6.6 cm, 15.0 cm, and 31.1 cm respectively. Whereas the growth-unit GU1 was formed before planting, the growth-unit GU2 was developped just after planting, from month 1 to 3; its short length was indicative of the planting stress.

Girth and height followed an initial fast increasing trend until month 17/18 (figure 12). Then a regular alternation of fast and slow growth was observed for girth, depending on the rainy and dry seasons. By contrast, a slow growth in height was observed along the whole period from 17 to 47 months, even in the rainy season R3.

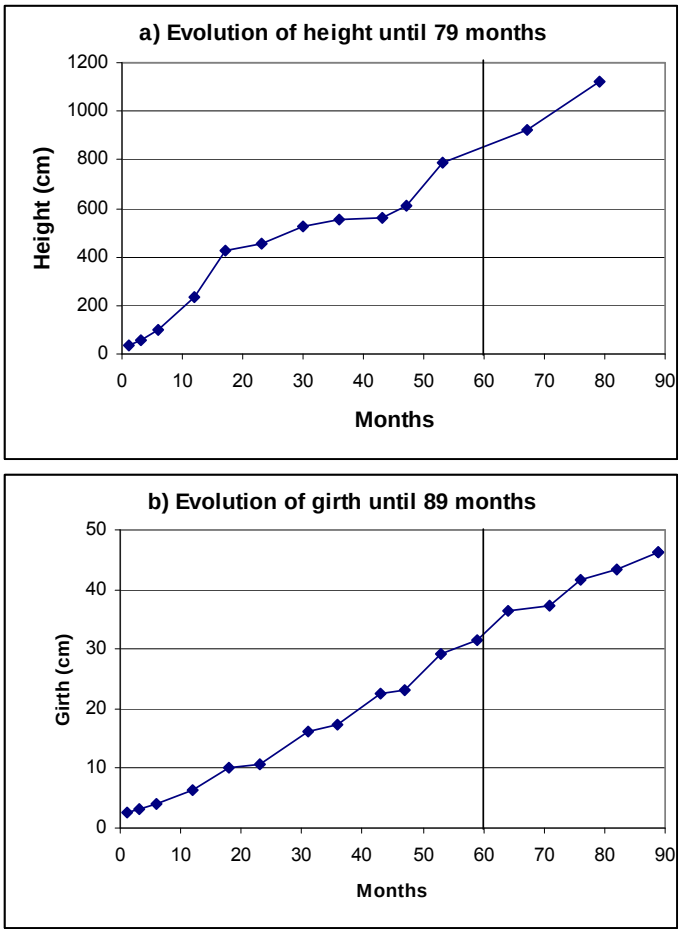
Table 8 : General data about the evolution of growth in height from month 1 to 79, issued from the BLUP estimations (cm). The range of variation goes from the lowest to the highest height. Coefficients of variation (CV %).

Months	Heights	Mean (cm)	Range (cm)	CV%
1	H1	33	14	8
3	H3	54	18	7
6	H6	103	29	6
12	H12	234	36	3
17	H17	425	108	5
23	H23	452	117	5
30	H30	524	129	5
36	H36	554	120	4
43	H43	559	85	3
47	H47	609	128	4
53	H53	789	181	4
67	H67	923	155	3
79	H79	1122	171	3
Season	Height increments			
R1	Hi13	21	17	14
R1	Hi36	49	19	7
D1	Hi612	131	26	4
R2	Hi1217	192	84	8
D2	Hi1723	27	41	29
R3	Hi2330	72	62	16
D3	Hi3036	30	83	41
R4	Hi3643	5	73	267
D4	Hi4347	50	90	28
R5	Hi4753	180	151	13
D5-R6	Hi5367	134	178	20
D6-R7	Hi6779	199	164	14

Table 9 : General data about growth in girth from month 1 to 89, issued from the BLUP estimations (cm). Ga = girth measured at 0.1 m high ; G = average of girths measured at 1.0 and 1.7 m high (cm). Coefficients of variation (CV %).

Month	Girths	Mean	Range	CV %
1	Ga1	1.6	0.4	4
3	Ga3	2.5	0.6	5
6	Ga6	3.9	0.5	2
12	Ga12	7.1	1.0	2
18	Ga18	12.6	3.2	5
23	Ga23	13.7	3.2	4
18	G18	10.1	2.2	4
23	G23	10.8	2.1	4
31	G31	16.3	2.4	3
36	G36	17.4	2.5	3
43	G43	22.6	2.4	2
47	G47	23.1	3.6	3
53	G53	29.1	4.8	3
59	G59	31.5	5.1	3
64	G64	36.3	8.3	4
71	G71	37.3	8.6	4
76	G76	41.5	11.4	5
79	G79	42.9	11.9	5
82	G82	43.4	11.7	5
85	G85	44.7	13.2	5
89	G89	46.2	14.3	6
Season	Girth increments			
R1	Gai13	0.9	0.5	10
R1	Gai36	1.4	0.3	4
D1	Gai612	3.2	0.7	4
R2	Gai1218	5.5	2.7	9
D2	Gai1823	1.1	1.2	18
D2	Gi1823	0.7	1.3	26
R3	Gi2331	5.5	1.0	3
D3	Gi3136	1.1	1.6	17
R4	Gi3643	5.2	1.8	6
D4	Gi4347	0.5	2.1	63
R5	Gi4753	6.0	2.4	7
D5	Gi5359	2.4	2.1	13
R6	Gi5964 (T07)	4.8	4.4	18
D6	Gi6471 (notap)	1.0	1.9	27
R7	Gi7176 (T08)	4.2	3.8	16
D7a	Gi7679 (notap)	1.4	3.2	25
D7b	Gi7982 (notap)	0.5	1.0	24
R8a	Gi8285 (T09)	1.3	2.6	27
R8b	Gi8589 (T09)	1.5	1.6	18

Figure 12 : Evolution of height (a) and girth (b) before and after the beginning of tapping. Tapping from month 60 to 64 in 2007, from month 72 to 75 in 2008, and from month 82 to 88 in 2009.



Figures 13 and 14 show the monthly height and girth increment rates of the successive periods, with the corresponding monthly rainfalls. Growth in the first rainy season (R1) was slower than growth in the subsequent dry season (D1), which may be due to the very young age of the trees and to the planting stress. During D1, monthly rainfall was higher than during all the subsequent dry seasons D2, D3, D4, and D5, and water requirements were still low for the small trees in D1. The fast growth in girth of the rainy periods p23-31 and p36-43 was contrasting with the slow growth in height for the same periods. Whereas growth in girth depends directly on the conditions of the period of growth, growth in height would depend also on the impact of the preceding period on the terminal buds.

Figure 13 : Monthly height increments (cm/month) of the 10 successive growth periods from month 1 to 53, and corresponding monthly rainfall (mm).

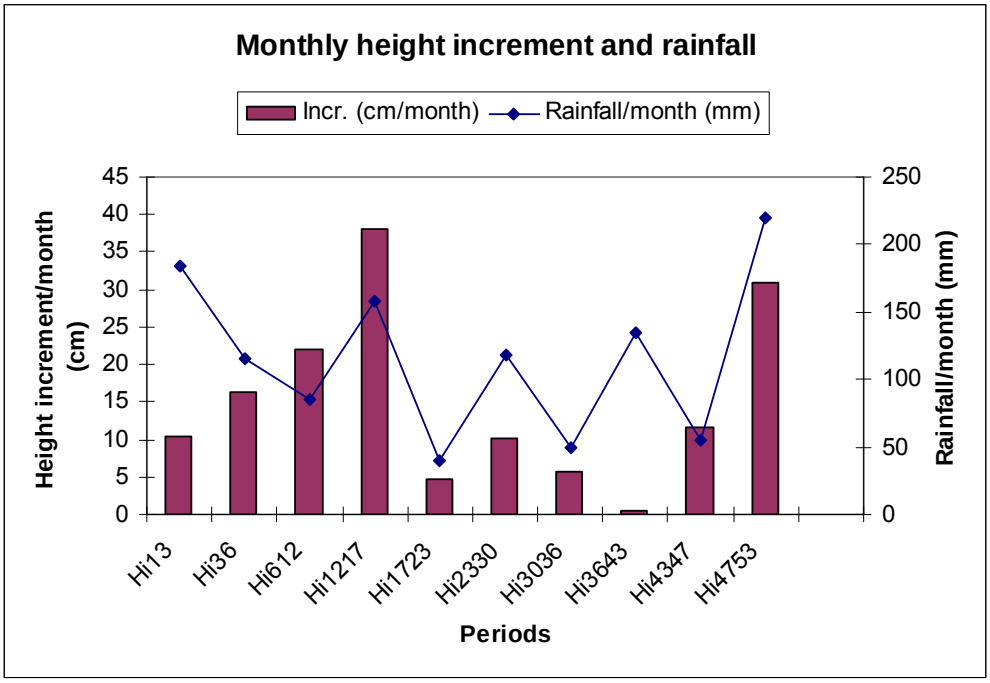


Figure 14 : Monthly girth increments at 1-m high (cm/month) of the 11 successive growth periods from month 1 to 59, and corresponding rainfall (mm).

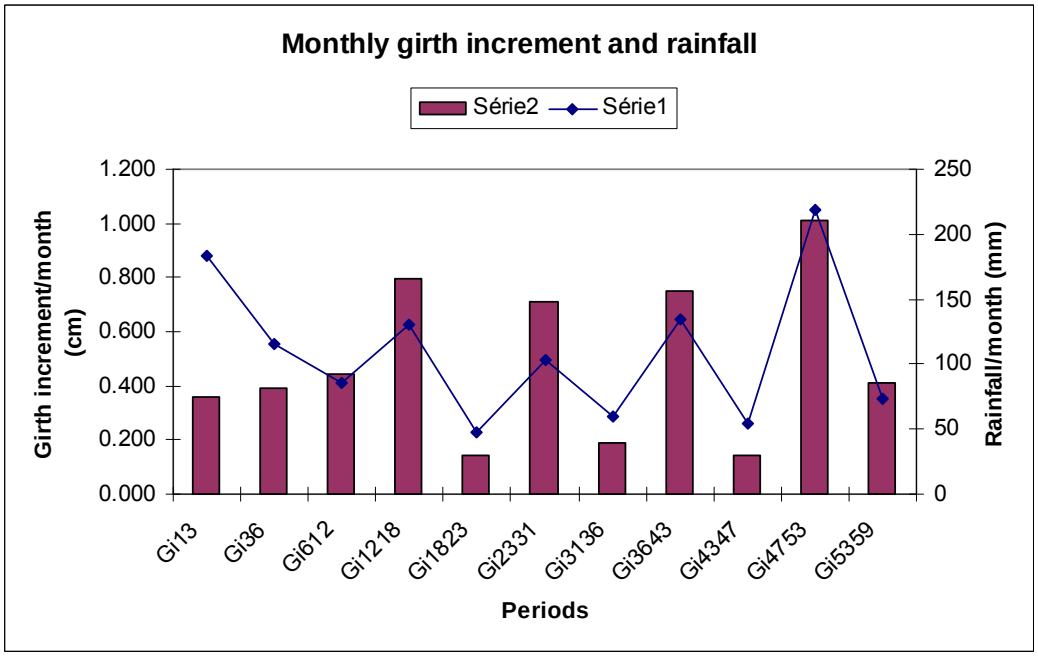
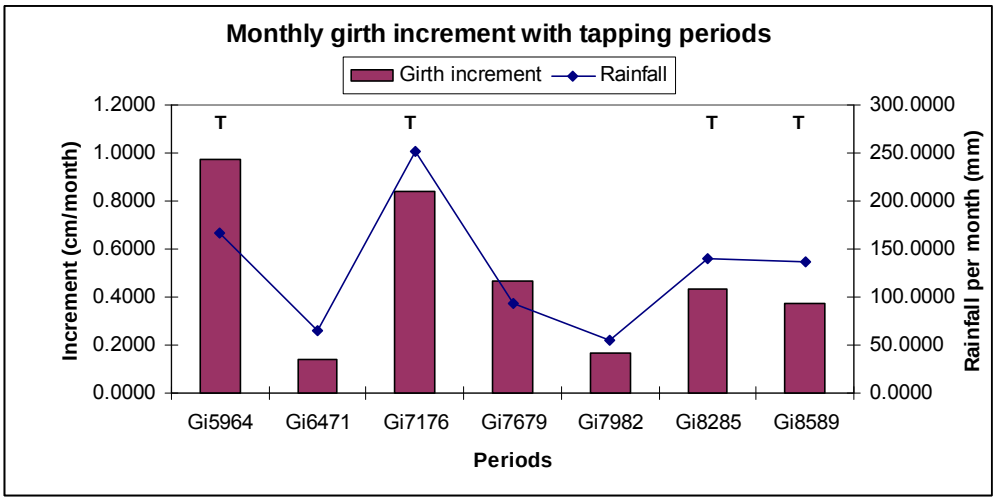


Figure 15 shows that growth in girth during tapping related with Gi5964 and Gi7176 were relatively high during these low-intensive tapping periods (2007 and 2008). By contrast, the growths related with Gi8285 and Gi8589 were lower due to intensive tapping and stimulation in 2009. During the dry season D7, the growth in girth of the first part of the season (Gi7679) was higher than the growth of the second part (Gi7982) due to lower rainfall and defoliation in this second part.

Figure 15 : Monthly girth increments at 1-m high (cm/month) of the growth periods since tapping initiation, and corresponding rainfall (mm). T = periods with tapping.



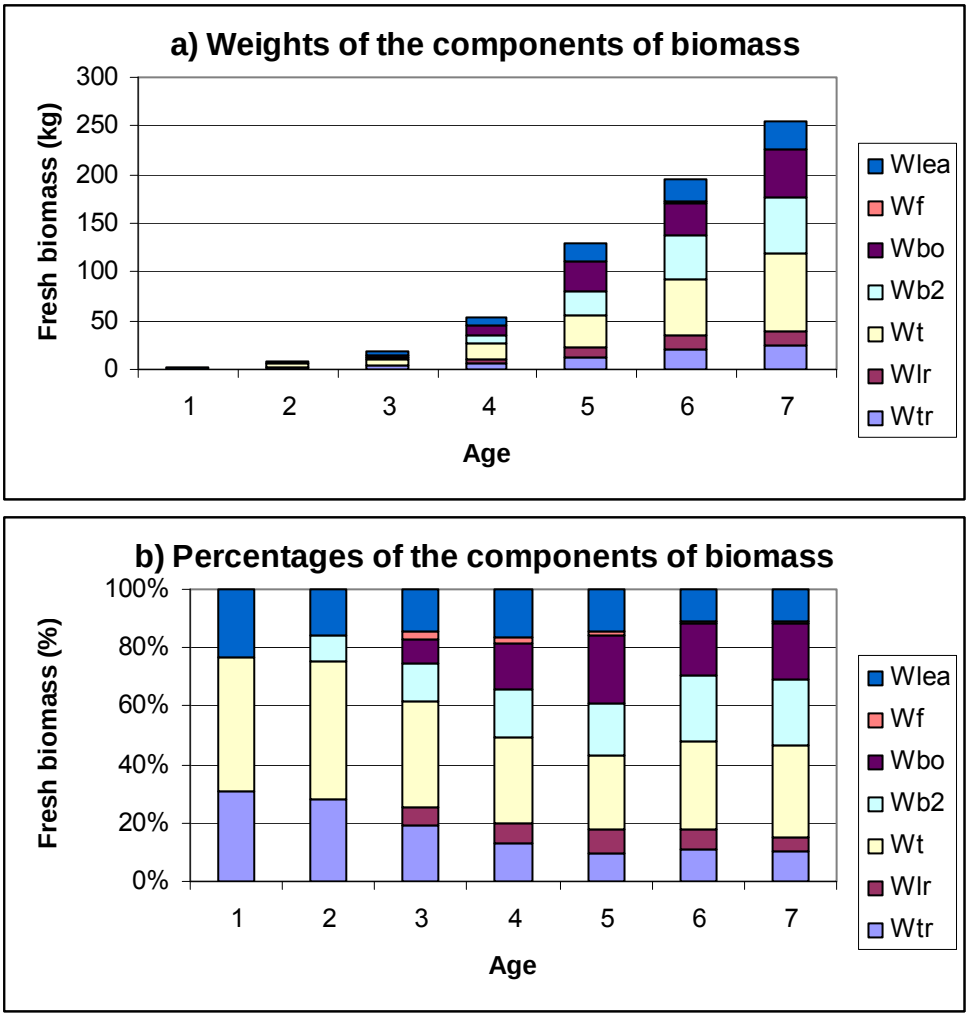
3.2.1.2. Evolution of biomass

The total fresh biomass of untapped trees at 7 years of age was of around 255 kg/tree, with a girth of 44 cm-large and a height of 10.5 m-high (Table 10). Figures 16-a and 16-b show the evolution of the fresh weights of the different components of biomass. Along time, the share of the trunk and of the roots decreased whereas the share of the branches increased.

Table 10 : Evolution of the average values of biomass-related traits from 1 to 7 years of age. G1 and H in cm, fresh weights in kg, no unit for the number of fruits (Nf).

Age	G1	H	Wtr	Wlr	Wt	Wb2	Wbo	Nf	Wf	Wlea	Twtree
1	5.2	236	0.4	0.0	0.6	0.0	0.0	0.0	0.0	0.3	1.2
2	13.6	485	2.3	0.0	3.8	0.7	0.0	0.0	0.0	1.3	8.0
3	16.8	515	3.4	1.1	6.4	2.3	1.4	14.3	0.5	2.5	17.5
4	24.7	600	7.1	3.7	15.9	9.0	8.7	18.9	1.0	9.0	54.5
5	33.1	806	12.4	10.5	33.1	23.4	30.6	25.0	1.3	19.1	130.3
6	39.7	941	21.3	13.5	58.0	44.1	34.3	33.2	1.7	21.6	194.5
7	43.6	1053	25.5	13.3	80.1	57.6	48.6	22.0	1.1	28.4	254.6

Figure 16 : Evolution of the average values of biomass components from 1 to 7 years of age. a) in fresh weight, b) in percentage. From Wlea to Wtr : leaves, fruits, other branches (order 3, 4, etc.), branches of order 2, trunk, lateral roots, and taproot.



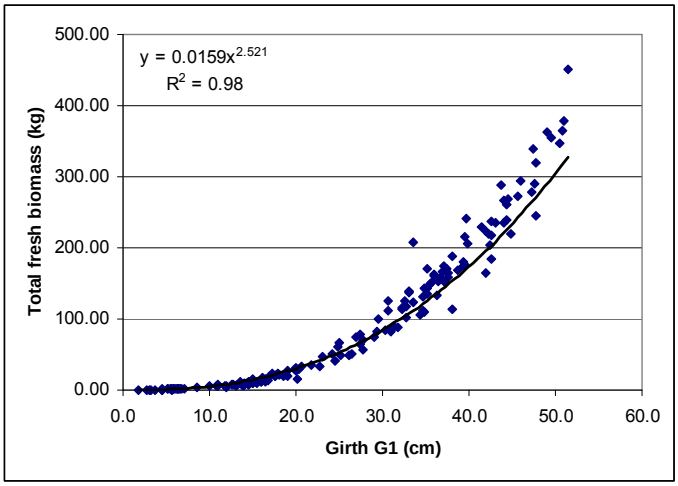
Each year, the explanation of the total weight (Tw) by the girth and the height was examined, based on multiple regression (table 11). Excepted for the first year, the girth G1 was always the predominant explaining factor.

Table 11 : Coefficients of determination and regression equations explaining the fresh total biomass from the girth G1 and the height H of the trees.

Age	R^2	Regression equations
1	0.89	Tw = - 0.64 + 0.18 H
2	0.79	Tw = - 5.37 + 0.95 G1
3	0.87	Tw = - 13.4 + 2.80 G1 - 3.12 H
4	0.93	Tw = - 66.7 + 5.08 G1
5	0.79	Tw = -169.2 + 9.3 G1
6	0.88	Tw = - 369.4 + 10.69 G1
7	0.87	Tw = - 439.7 + 14.84 G1

Figure 17 shows the evolution of the total fresh biomass with the girth measured at 1-m high over the 7-year period. Within the genetic framework of this family for which a good homogeneity of branching structure was observed among the progenies, girth appeared as a very good predictor of the fresh biomass of untapped trees.

Figure 17 : Evolution of the total fresh biomass with girth.



The equation predicting the biomass from the girth was used in the trial for converting the BLUPs of girth into biomass. Table 12 shows the percentages of total biomass gained in each of the 12 successive growth periods (months) until 59

months, the cumulated rainfall and the rainfall per month. Most of biomass acquisition was done during the rainy seasons R2, R3, R4, and R5, and the dry season D5, representing 32 months equivalent to 54 % of the total period and 92 % of the total biomass.

Table 12 : Growth in biomass over 12 successive periods from month 0 to month 59, and corresponding rainfall.

Periods	Season	%biomass	Rainfall (mm)	Rain/month (mm)
0-1	R1	0.04	131	131
1-3	R1	0.07	367	184
3-6	R1	0.22	345	115
6-12	D1	1.12	514	86
12-18	R2	4.75	789	132
18-23	D2	1.06	235	47
23-31	R3	12.58	827	103
31-36	D3	2.91	299	60
36-43	R4	20.69	946	135
43-47	D4	2.82	217	54
47-53	R5	35.66	1315	219
53-59	D5	18.09	436	73

3.2.1.3. Heritabilities

Table 13 shows the heritabilities of the growth traits. They were of a low level, thus indicating a low genetic variability (h^2l from 0.03 to 0.27). However, heritabilities of girth were higher after the beginning of tapping. Although the effect of age might be suspected, it can be thought that the new genetic influence of latex production on girth increment generated this increase in the heritability of girth traits.

Table 13 : Heritabilities of height and girth traits.

Height	h²l		Girth	h²l
H1	0.18		Ga1	0.13
H3	0.09		Ga3	0.08
H6	0.06		Ga6	0.03
H12	0.04		Ga12	0.04
			Ga18	0.13
			Ga23	0.11
H17	0.11		G18	0.13
H23	0.12		G23	0.10
H30	0.20		G31	0.09
H36	0.17		G36	0.08
H43	0.16		G43	0.07
H47	0.15		G47	0.08
H53	0.18		G53	0.13
			G59	0.14
Tapping initiation				
			G64	0.21
H67	0.17		G71	0.20
			G76	0.25
H79	0.15		G79	0.25
			G82	0.25
			G85	0.26
			G89	0.27

3.2.1.4. Correlations

There were positive correlations between nearly all the couples of height traits taken from H1 to H79. The coefficients of correlation varied from 0.11 (non significant) to 0.93, with an average of r = 0.47. Similarly, there were positive correlations between nearly all the couples of girth traits taken from Ga1 to G89. The coefficients of correlation varied from 0.13 (non significant) to 1.00, with an average of r = 0.61.

Table 14 shows the correlations between girth and height traits of the same age, or girth and height increments of the same growth period. In most cases, there were positive correlations. However the correlation between Gai36 and Hi36 was low (r = 0.15). Whereas the coefficients of correlation were high for traits from month 1 to 18/17, a decrease was observed from month 18/17 to month 43, corresponding to the drought period. Moreover there was no correlation between girth increment and

height increment for the periods D2, R3, and D4 before tapping, and for the periods D5-R6 and D6-R7-D7 after tapping initiation.

Table 14 : Coefficients of correlation between girth and height for the same dates or periods of increments (df = 194 ; significance threshold r = 0.14 for α = 0.05).

Girth	Height	r	Month
Ga1	H1	0.67	1
Ga3	H3	0.76	3
Ga6	H6	0.79	6
Ga12	H12	0.84	12
G18	H17	0.73	18/17
G23	H23	0.65	23
G31	H30	0.61	31/30
G36	H36	0.49	36
G43	H43	0.36	43
G47	H47	0.39	47
G53	H53	0.51	53
G64	H67	0.43	64/67
G71	H67	0.43	71/67
G76	H79	0.49	76/79
G79	H79	0.51	79
G82	H79	0.50	82/79
G85	H79	0.50	85/79
G89	H79	0.50	89/79
			Season
Gai13	Hi13	0.66	R1
Gai36	Hi36	0.15	R1
Gai612	Hi612	0.64	D1
Gai1218	Hi1217	0.64	R2
Gi1823	Hi1723	ns	D2
Gi2331	Hi2330	ns	R3
Gi3136	Hi3036	0.36	D3
Gi3643	Hi3643	0.20	R4
Gi4347	Hi4347	ns	D4
Gi4753	Hi4753	0.27	R5
Gi5364	Hi5367	ns	D5-R6
Gi6479	Hi6779	ns	D6-R7-D7

The study of the correlations between pairs of growth increments (girths and/or heights) over the successive periods of rainy and dry seasons showed a wide variety of cases with no correlation, negative correlations, or positive correlations, thus indicating varying responses of the genotypes to the variations of the environment.

As far as H53 and G59 indicate the final result of growth after five years, the correlations between the growth increments of the successive periods and the final traits H53 and G59 were examined. (table 15). Positive correlations were found for the seasons D1, R2, R3, D4, R5, and D5, thus indicating that the genotypes followed the general growth trend during these periods. By contrast, non significant correlations were found for the seasons R1, D2, D3, and R4. Moreover, the increments of the season D3 were negatively correlated with H53. During these seasons, the genotypes provided responses to the environment different from the general growth trend.

Table 15 : Correlations between height and girth increments of the successive growth periods and the final height H53 and girth G59 (df = 194, significance threshold r = 0.140 for α = 0.05).

Season	Traits	H53	G59
R1	H1	ns	0.14
	Ga1	ns	0.31
R1	Hi13	ns	ns
	Gai13	ns	0.14
R1	Hi36	0.16	0.17
	Gai36	ns	ns
D1	Hi612	0.27	0.25
	Gai612	0.33	0.46
R2	Hi1217	0.49	0.43
	Gai1218	0.39	0.64
D2	Hi1723	ns	ns
	Gai1823	ns	ns
	Gi1823	ns	ns
R3	Hi2330	0.42	0.21
	Gi2331	0.24	0.41
D3	Hi3036	-0.24	ns
	Gi3136	-0.17	ns
R4	Hi3643	ns	-0.15
	Gi3643	ns	ns
D4	Hi4347	0.21	ns
	Gi4347	0.18	0.56
R5	Hi4753	0.68	0.29
	Gi4753	0.41	0.76
D5	Gi5359	0.29	0.64

Table 16 shows the relationships between the girth increments in the rainy seasons from R2 to R8, before tapping and during tapping periods. In all cases, there were positive correlations between girth increments during the tapping

periods, thus suggesting that the genotypes behaved similarly for growth during every tapping period, in response to tapping and latex production. Moreover, in most cases there were negative correlations between these girth increments and the production traits.

However the girth increment of 2008 (Gi7176) was not correlated with the production of 2009, and the two girth increments of 2009 (Gi8285 and Gi8589) were not correlated with the productions of 2008 and 2009. This suggests a physiological change in 2009, associated with the intensification of tapping. Probably the girth increments in 2009 have reached a minimum which was out of the usual negative linear relationship with the production. The two girth increments Gai1218 and Gi4753, corresponding to the main growth trend, were positively correlated with the girth increments during tapping, and also with the production of 2009.

Table 16 : Correlations between girth increments in the rainy seasons from R2 to R8, before tapping and during tapping periods. Relationships with the productions Lp2007, Lp2008, and Lp2009. Df = 194, threshold r = 0.14 for α = 0.05. The mean girth increments were also indicated (cm).

	Season	R2	R3	R4	R5	R6	R7	R8a	R8b
Traits	Year	Gai1218	Gi2331	Gi3643	Gi4753	Gi5964	Gi7176	Gi8285	Gi8589
	Mean (cm)	5.47	5.52	5.16	5.98	4.85	4.20	1.30	1.50
Gai1218	2003	1.00	0.14	-0.25	0.48	0.36	0.26	0.23	0.20
Gi2331	2004	0.14	1.00	ns	0.17	ns	ns	ns	ns
Gi3643	2005	-0.25	ns	1.00	ns	ns	ns	ns	ns
Gi4753	2006	0.48	0.17	ns	1.00	0.40	0.43	0.36	0.38
Gi5964	2007	0.36	ns	ns	0.40	1.00	0.68	0.52	0.42
Gi7176	2008	0.26	ns	ns	0.43	0.68	1.00	0.60	0.52
Gi8285	2009a	0.23	ns	ns	0.36	0.52	0.60	1.00	0.60
Gi8589	2009b	0.20	ns	ns	0.38	0.42	0.52	0.60	1.00
Lp2007		ns	ns	ns	ns	-0.42	-0.28	-0.14	-0.14
Lp2008		ns	ns	ns	ns	-0.39	-0.31	ns	ns
Lp2009		0.14	ns	ns	0.24	-0.14	ns	ns	ns

3.2.2. Latex production (2007-2009)

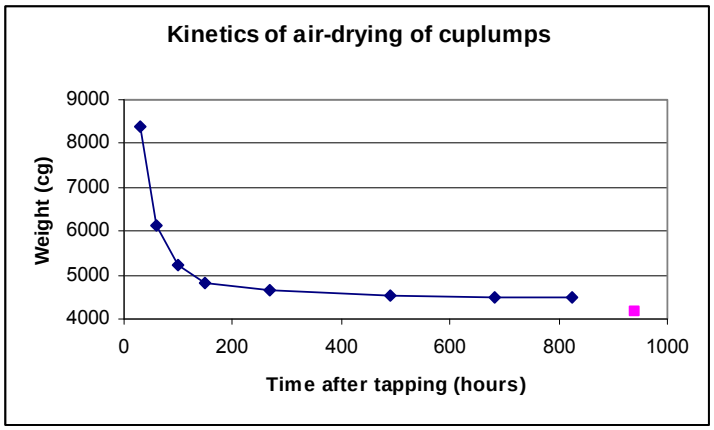
3.2.2.1. Correlation between cuplumps and dry rubber

Latex production data were based on the weighing of coagulated cuplumps and not on completely dry rubber weight. For justifying this method, the kinetics of air-drying of the cuplumps was studied on 200 cuplumps which were collected from 200 trees after tapping on May 28, 2009. This sample was made of 65 different genotypes, with from 1 to 4 trees per genotype. Air-drying was carried out for one month until July 1, and drying was completed in owen from July 1 to July 6. Nine series of weighings, from W1 to W8 and Wdry, were made at regular intervals (table 17 and figure 18).

Table 17 : Kinetics of cuplumps air-drying over a period of one month. Correlations of each series of weighing with the final dry weight.

Day	Weighing	Time (h)	Nb days	Min (cg)	Max (cg)	Average (cg)	r (corr with Wdry)
29 May	W1	29	1.2	1027	23206	8377	0.967
30 May	W2	58	2.4	625	18098	6133	0.977
01 June	W3	99	4.1	602	15239	5210	0.993
03 June	W4	149	6.2	589	13351	4817	0.999
08 June	W5	269	11	581	12843	4650	1.000
17 June	W6	491	20.5	568	12629	4549	1.000
25 June	W7	683	28.5	560	12553	4510	1.000
01 July	W8	825	34.4	562	12545	4508	1.000
06 July	Wdry	940	after oven	562	10832	4171	1.000

Figure 18 : Kinetics of cuplumps air-drying over a period of one month (Wdry measured after 940 hours).



From these data, it was shown that the average percentage of dry rubber for 10 cuplumps collected every three days from one same tree over one month varied from 82 to 88 %. This high level of air-drying over one month, the high correlations between cuplump weighing and dry rubber weighing, and the fact that all the trees were submitted to the same schedule of cuplump collection show that data issued from the weighing of cuplumps cumulated over one month were highly correlated with what would have been obtained with dry rubber.

3.2.2.2. Evolution of latex production

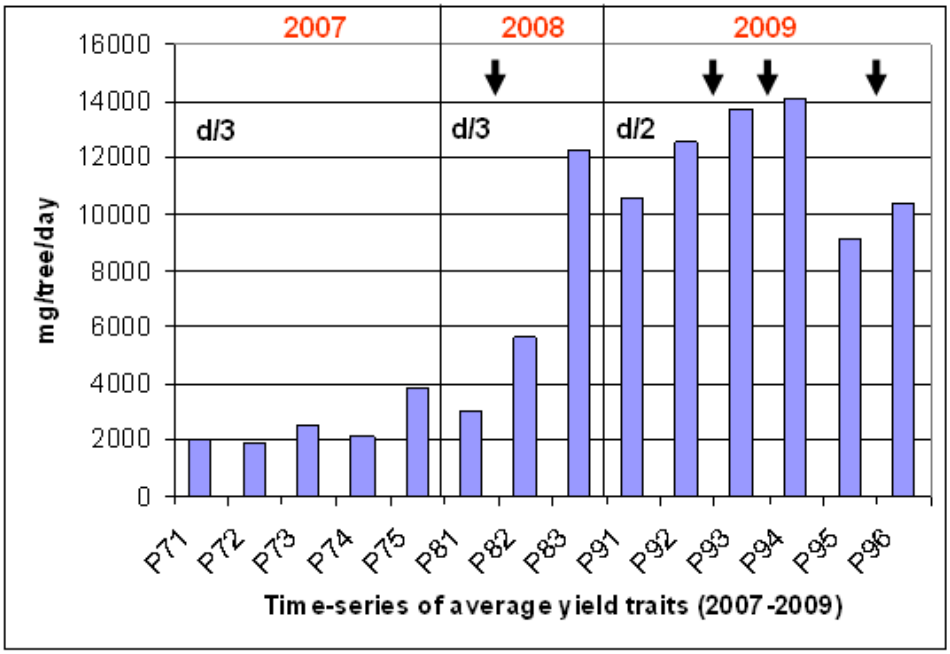
Table 18 gives the number of trees measured individually, and the general means of the traits, for girth prior to tapping in 2007, 2008, and 2009, and for rubber productions in 2007, 2008, and 2009. In 2007, for the traits P71 to P75, tapping was in d/3 (every three days) with no stimulation (average girth of 32.3 cm), and yield was low : it varied from 1909 mg/tree/day (P72) to 3847 mg/tree/day (P75). This level was still observed for the first yield trait P81 (3006 mg/tree/day) that was measured before the application of the first stimulation in 2008. After the application of 20 mg of ethephon per tree on 15 July 2008, yield was only slightly increased for P82 (5623 mg/tree/day) due to the many tappings canceled by rain. This trait P82 should be considered as under-estimated. Then an important increase in yield was observed for P83 (12267 mg/tree/day), due to resuming of regular tapping and to a long-lasting effect of the stimulation. In 2009, tapping was in d/2 (every two days). A first stimulation with a low concentration (5 mg/tree) was applied at the initiation of tapping (before P91) ; its effect was probably very low and it was considered as non-significant in data examination. Three stimulations (25 mg/tree) were applied before the tapping periods corresponding to P93, P94, and P96. Yield levels were higher than 10000 mg/tree/day for P91, P92, P93, P94, and P96, and slightly lower than this level for P95 (9085 mg/tree/day).

Table 18 : Girth before tapping (G59 in May 2007, G71 in May 2008, and G82 in April 2009), and production in 2007 (P71-P75), 2008 (P81-P83), and 2009 (P91-P96).

Year	Trait	Nb trees	Mean	Unit
2007	G59 (G7)	2283	32.3	cm
2008	G71 (G8)	2279	38.8	cm
2009	G82 (G9)	2278	45.5	cm
2007	P71	2283	2040	fresh mg/tree/day
	P72	2284	1909	fresh mg/tree/day
	P73	2283	2485	fresh mg/tree/day
	P74	2266	2146	fresh mg/tree/day
	P75	2283	3847	fresh mg/tree/day
2008	P81	2259	3006	fresh mg/tree/day
	P82	2270	5623	fresh mg/tree/day
	P83	2255	12267	fresh mg/tree/day
2009	P91	2244	10579	fresh mg/tree/day
	P92	2244	12526	fresh mg/tree/day
	P93	2242	13692	fresh mg/tree/day
	P94	2198	14112	fresh mg/tree/day
	P95	2160	9085	fresh mg/tree/day
	P96	2120	10375	fresh mg/tree/day

Figure 19 shows the evolution of the average yield along the time-series of yield traits from P71 to P96. From this evolution, two phases can be considered : a) a low-intensive phase from P71 to P82 with a low production, b) an intensive phase from P83 to P96 with a high production. The final part of the intensive phase showed a slightly depressed production from P95 to P96 probably due to physiological limitations appearing along the intensive phase. The depressed production of P95 and the poor response to stimulation of P96 probably indicate that the trees had reached their limit after the productions P93 and P94.

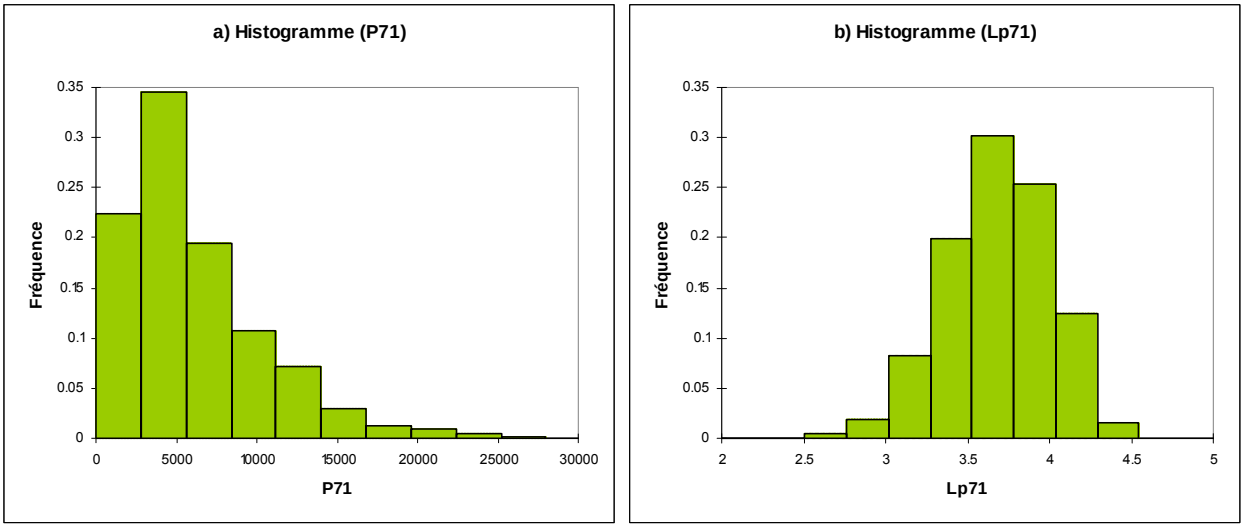
Figure 19 : Evolution of average yields (mg/tree/day) along the time-series of yield traits from P71 (June 2007) to P96 (October 2009). Arrows indicate the four stimulations before P82 in 2008, and before P93, P94, and P96 in 2009. Tapping frequency : d/3 from P71 to P83, d/2 from P91 to P96.



3.2.2.3. Distribution of the production data

Normality of the distributions of production data was tested from the values of skewness and kurtosis, and from the Kolmogorov-Smirnov hypothesis test (KS). The phenotypic values of rubber production traits were not normally distributed but positively skewed, or right-skewed (with a long tail on the right side). Therefore, data were normalized by a log transformation : $Y = {}^{10}\log(X)$. Figure 20 shows the effect of the log transformation for the trait P71 (first cumulated rubber production measured in June 2007).

Figure 20 : Distributions of latex yield P71 and of the transformed trait Lp71 = ^{10}log(P71) ; a) skewness = 1.40 , kurtosis = 2.24, Kolmogorov-Smirnov test statistic KS = 0.118 ; b) skewness = -0.32 , kurtosis = -0.05 , KS = 0.023.



The BLUP estimations of the genetic values of the genotypes were estimated based on the transformed data, first with no adjustement to the girth of the trees, and then after girth adjustment (the girth was introduced in the model as a covariable playing the role of one more fixed effect).

The coefficients of variation of the BLUPs (CV%) varied from 3.2 to 4.8 % for the three girth traits (G7, G8, G9), and from 17.9 to 57.6 % for the yield traits, so showing a variability much higher for production than for growth.

3.2.2.4. Heritabilities and correlations

Table 19 shows the correlations between on the one hand the BLUPs of girth, and on the other hand the BLUPS of the production traits. The three girths G7, G8, and G9 were highly correlated between each other. All the yield traits were significantly correlated with G7, and the level of the correlation was increased from Lp92 to Lp94. The girth traits G8 and G9 were not correlated with the yield traits from Lp71 to Lp91, due to growth modifications induced by latex production. However G8 and G9 were correlated with the production from Lp92 to Lp96. This indicates that a change in the genetic determinism of latex production from Lp92 to Lp96 occured since May or June 2009 : production became more dependent on the size of the

genotypes which can be considered as a limiting factor of the production. This effect was visible since Lp92, therefore prior to the first stimulation which was applied after Lp92 and before LP93. This effect was still stronger for the stimulated Lp93 (after the first stimulation in 2009), but it decreased afterwards, thus indicating another modification in the genetic determinism of the production, probably due to the effect of other limiting factors.

Taking into account the positive correlations between G7 and all the production traits, G7 was chosen as a covariable and added to the fixed effects of the statistical model for estimating girth-adjusted production BLUPs of the genotypes. Thus the girth-adjusted production traits were considered at « similar level of the girth G59 », just as if the trees were all opened at the same size, which is actually the case in industrial tapping. The names of the traits, for this second set of girth-adjusted production traits, were from Lp71a to Lp96a.

The heritabiity of production (h^2l-1) was high from Lp71 to Lp91, but it decreased afterwards, thus indicating a reduction in genetic variability. Table 19 shows that the heritabilities (h^2l-2) were increased thanks to the adjustment on girth. Each year, the first production trait of the year showed the highest heritability (Lp71 in 2007, Lp81 in 2008, and Lp91 in 2009).

Table 19 : Correlations between girth and yield traits (BLUPs) over the three years 2007, 2008, and 2009 (df = 194, significant threshold r = 0.14 for α = 0.05). In bold : significant coefficients of correlation between on the one hand G8 and G9, and on the other hand yield traits from Lp92 to Lp96. Heritabilities of the yield traits, before and after adjustment on G7 (h^2l-1 and h^2l-2 respectively).

Traits	G7	G8	G9	h^2l-1	h^2l-2
G7	1	0.93	0.86	0.14	-
G8	0.93	1	0.97	0.22	-
G9	0.86	0.97	1	0.27	-
Lp71	0.26	0.05	0.01	0.59	0.71
Lp72	0.25	0.04	0.02	0.49	0.59
Lp73	0.24	0.03	-0.01	0.49	0.62
Lp74	0.16	-0.04	-0.06	0.47	0.54
Lp75	0.24	0.04	0.01	0.40	0.47
Lp81	0.15	-0.02	-0.05	0.56	0.62
Lp82	0.21	0.04	0.01	0.39	0.42
Lp83	0.22	0.05	0.02	0.48	0.55
Lp91	0.19	0.02	0.00	0.46	0.50
Lp92	0.35	**0.22**	**0.21**	0.31	0.35
Lp93	0.51	**0.45**	**0.46**	0.22	0.23
Lp94	0.42	**0.34**	**0.34**	0.29	0.32
Lp95	0.25	**0.16**	**0.19**	0.33	0.35
Lp96	0.25	**0.17**	**0.20**	0.26	0.29

The coefficients of correlation between all the girth-adjusted production traits varied from 0.54 to 0.94 (mean r = 0.75). Thus there were strong positive correlations between all pairs of traits among the three years, and no important modification in the ranking of the genotypes along the process of intensification.

3.2.3. Plugging index

3.2.3.1. Average values of the traits

There were two series of measurements of the plugging index PI in 2007, corresponding to the periods of production P73 and P74 (low-intensive period), and one series in 2009 corresponding to P96, at the end of the intensive tapping period. The traits W1 (first part of production of the first five minutes after tapping) and W2 (second part of production after the first five minutes) were normalized by a log-transformation. The traits PI73 and PI74 were also normalized by a log-

transformation, but it was not necessary for PI96 (normal distribution of natural data). Drc was measured only on the two series of 2007.

Table 20 shows the mean values of W1, W2, WT (W1+W2), Drc, and PI (plugging index) for the three series corresponding to P73, P74, and P96. By comparison with the two series measured in 2007, the series measured in 2009 showed a lower W1, and a higher W2, which was waited after tapping intensification. Among the genotypes, whereas PI73 and PI74 varied from 8 % to 53 %, PI96 varied only from 6 % to 11 %.

In 2009, tapping frequency was passed from d/3 to d/2. This explains why W1 was lower in 2009 : there was a lower turgor pressure in the laticifers due to a higher tapping frequency and a shorter regeneration period. Concerning W2, as a consequence of the 3 stimulations in 2009, latex flow was much longer in October 2009 (P96) than in 2007, and W2 was higher, thus generating a decrease in the value of the plugging index. Moreover, the variability between the genotypes was considerably reduced for W2 after intensification.

Table 20 : Mean BLUP values of W1, W2, WT (W1+W2), Drc, and PI (plugging index), minimum and maximum for the three series corresponding to P73, P74, and P96.

Traits	P73 series	P74 series	P96 series
W1 (cg)	426	391	236
Min W1 (cg)	178	180	140
Max W1 (cg)	755	643	330
W2 (cg)	1332	1426	2633
Min W2 (cg)	327	272	2160
Max W2 (cg)	4126	5272	3074
WT (cg)	1758	1817	2869
Drc %	39.9	41.0	-
PI %	25.0	22.7	8.3
Min PI %	9.7	8.7	5.8
Max PI %	52.7	52.5	10.3

3.2.3.2. Heritabilities

Plugging indexes (PI73, PI74, and PI96) were calculated directly from the BLUPs of W1 and W2 traits. Thus heritability was not estimated for PI traits. Table 21 shows a very low heritability for DrcPI73 (h^2I = 0.06).

By comparison with the year 2007, the heritability of W1 was decreased in 2009, and the heritability of W2 was strongly decreased, thus indicating a reduction in genetic variability, notably for W2.

Table 21 : Heritabilities of the traits W1 and W2 for the three series measured in 2007 and 2009.

Year	Series	Type	Trait	h^2I
2007	1	W1	LW173	0.46
		W2	LW273	0.48
		Drc	DrcPI73	0.06
	2	W1	LW174	0.44
		W2	LW274	0.54
2007	3	Drc	DrcPI74	0.24
2009	3	W1	LW196	0.20
		W2	LW296	0.07

3.2.3.3. Correlations

Table 22 shows the correlations between the traits of the three series of plugging index measurement. There were positive correlations between the traits of the same type (LW1, LW2, Drc, or PI). In most cases, the traits LW1 and LW2 were positively correlated between each other, and the LW2 traits were negatively correlated with Drc and PI traits. Drc traits were positively correlated with PI traits. Whereas PI73 and PI74 were strongly and negatively correlated with LW273 and LW274 respectively, and not correlated with LW173 and LW174, PI96 was strongly and positively correlated with LW196, and not correlated with LW296. This was a consequence of the reduction of variability of W2 under stimulated tapping. There was no correlations between W1 and Drc for the two series measured in 2007.

Table 22 : Correlations between the « plugging index » traits of the three series measured in 2007 and 2009 (df = 194 ; threshold r = 0.14 for α = 0.05).

Traits	LW173	LW174	LW196	LW273	LW274	LW296	DrcPI73	DrcPI74	LPI73	LPI74	LPI96
LW173	1.00	0.83	0.55	0.42	0.31	0.39	ns	ns	Ns	ns	0.45
LW174	0.83	1.00	0.50	0.35	0.36	0.36	ns	ns	ns	ns	0.41
LW196	0.55	0.50	1.00	ns	ns	0.51	ns	0.22	ns	0.23	0.91
LW273	0.42	0.35	ns	1.00	0.89	0.39	-0.36	-0.48	-0.36	-0.47	-0.22
LW274	0.31	0.36	ns	0.89	1.00	0.37	-0.32	-0.55	-0.32	-0.55	-0.27
LW296	0.39	0.36	0.51	0.39	0.37	1.00	ns	ns	ns	ns	ns
DrcPI73	ns	ns	ns	-0.36	-0.32	ns	1.00	0.27	1.00	0.27	ns
DrcPI74	ns	ns	0.22	-0.48	-0.55	ns	0.27	1.00	0.27	1.00	0.30
LPI73	ns	ns	ns	-0.36	-0.32	ns	1.00	0.27	1.00	0.27	Ns
LPI74	ns	ns	0.23	-0.47	-0.55	ns	0.27	1.00	0.27	1.00	0.30
LPI96	0.45	0.41	0.91	-0.22	-0.27	ns	ns	0.30	ns	0.30	1.00

Correlations between W2 and Drc were negative for the two series corresponding to P73 and P74. This complies with the fact that lower the Drc and longer the latex flow. But LW296 was not correlated with the two Drc traits. For better studying the relationships between W2 and Drc, we used the Drc traits issued from latex diagnostic measurements (table 23). LW296 was not correlated with most of the Drc traits. This was due to the fact that the genetic variability of LW296 was very low (low heritability, and low coefficient of variation between the BLUPs).

Table 23 : Correlations between LW2 traits and the Drc traits measured in latex diagnostic series (df = 194 ; threshold r = 0.14 for α = 0.05).

Traits	LW273	LW274	LW296
Drc72a	-0.44	-0.42	-0.17
Drc72b	-0.43	-0.46	ns
Drc74	-0.48	-0.49	ns
Drc81	-0.35	-0.38	ns
Drc82	-0.34	-0.38	ns
Drc92	-0.31	-0.32	ns
Drc93	ns	ns	ns
Drc95	-0.18	-0.22	ns
Drc96	ns	ns	ns

Table 24 shows a high level of correlation between on the one hand LW273 and LW274, and on the other hand the corresponding production traits Lp73a and Lp74a, thus confirming that latex production is strongly related with the duration of

latex flow, and only slightly related with the initial production of the first five minutes after tapping.

Table 24 : Correlations between the traits characterizing the plugging index and the corresponding production traits. Lines relative to LW2 traits in bold (df = 194 ; threshold r = 0.14 for α = 0.05).

Traits	Lp73a	Lp74a	Lp96a
LW173	0.290	0.226	0.274
LW273	**0.839**	**0.846**	**0.569**
LW174	0.296	0.254	0.250
LW274	**0.830**	**0.859**	**0.559**
LW196	ns	ns	0.156
LW296	**0.348**	**0.371**	**0.506**
LPI73	-0.731	-0.786	-0.468
LPI74	-0.285	-0.285	Ns
DrcPI73	-0.301	-0.307	-0.180
DrcPI74	-0.532	-0.555	-0.243
LPI96	-0.285	-0.285	ns

3.2.4. Latex diagnostic

3.2.4.1. General results issued from BLUP estimations

The nine series of latex diagnostic LD72a, LD72b, LD74, LD81, LD82, LD92, LD93, LD95, and LD96 were measured during the periods corresponding to the latex production traits P72 (two successive latex diagnostics LD72a and LD72b), P74, P81, P82, P92, P93, P95, and P96. Measurements were carried out on individual trees, with around 4 trees per genotype (one tree in each full replication of the experimental design). One same sample of 793 trees was used for the nine LD series. From the examination of the distributions of the LD traits, it was found that the sucrose traits required a root transformation for normalization of the distributions : Y = root(X). BLUPs and heritabilities of the traits were estimated.

Table 25 summarizes the general results issued from the BLUP estimations of the genotypes (mean, minimum, maximum, standard deviation, coefficient of variation). One of the most salient features of these tables was the decreasing trend of sucrose content and the increasing trend of Pi content over time, in relation with intensification.

Table 25 (a, b, c, d) : General results issued from BLUP estimations of the latex diagnostic traits (LD).

a) Dry rubber content (Drc, %)

Codec	Drc72a	Drc72b	Drc74	Drc81	Drc82	Drc92	Drc93	Drc95	Drc96
Mean	33.96	38.63	40.93	41.86	37.11	37.50	35.41	40.76	39.49
Min	28.67	31.32	31.95	34.30	30.16	31.78	33.66	36.26	36.31
Max	40.09	46.34	48.54	48.48	48.43	44.68	38.93	45.41	44.22
h^2l	0.29	0.38	0.37	0.36	0.35	0.32	0.08	0.15	0.10

The three last Drc observed in 2009 still had medium values, but their heritabilities were very low.

b) Sucrose (Suc, root transformed ; mM)

Codec	RSuc72a	RSuc72b	RSuc74	RSuc81	RSuc82	RSuc92	RSuc93	RSuc95	RSuc96
Mean	4.59	4.52	4.15	3.56	3.71	2.66	2.49	2.29	2.37
Min	3.69	3.95	3.00	2.97	3.00	2.29	2.36	2.16	2.12
Max	5.49	5.25	5.51	4.24	4.42	2.96	2.60	2.40	2.53
h^2l	0.21	0.17	0.35	0.16	0.14	0.09	0.03	0.02	0.04

The means of sucrose content followed a decreasing trend over the three years and the 9 LD series. The heritabilities also followed a decreasing trend, with very low levels for RSuc93, RSuc95, and RSuc96.

c) Inorganic phosphorus (Pi, mM)

Codec	Pi72a	Pi72b	Pi74	Pi81	Pi82	Pi92	Pi93	Pi95	Pi96
Mean	11.54	14.51	15.36	10.30	22.11	24.44	29.87	25.29	31.68
Min	6.39	8.51	8.42	4.17	10.95	13.23	18.09	19.27	19.21
Max	21.15	26.17	27.43	26.34	40.80	42.95	41.28	33.66	44.70
h^2l	0.43	0.44	0.22	0.53	0.50	0.42	0.39	0.24	0.31

The means of inorganic phosphorus content followed an increasing trend over the three years and the 9 LD series (with an irregularity for Pi81). The heritabilities were lower for Pi93, Pi95, and Pi96.

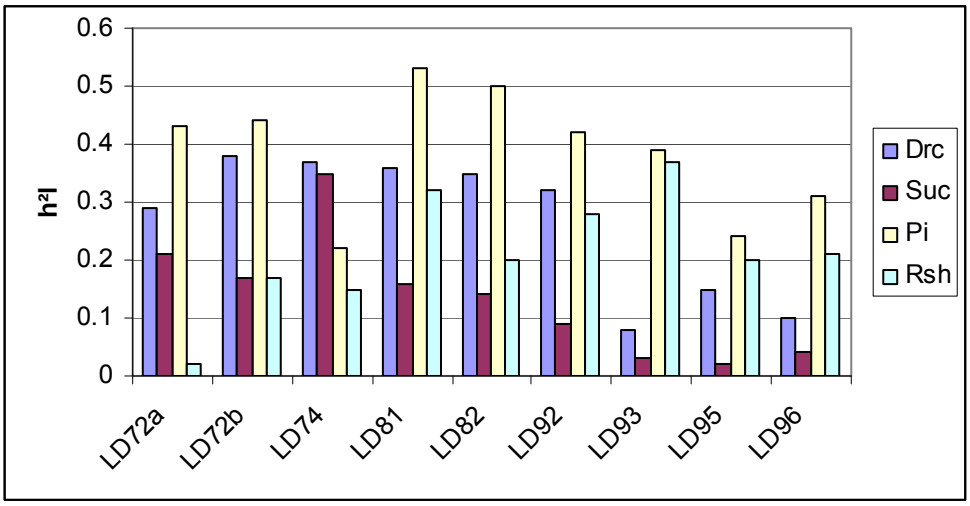
d) Thiols (Rsh, mM)

Codec	Rsh72a	Rsh72b	Rsh74	Rsh81	Rsh82	Rsh92	Rsh93	Rsh95	Rsh96
Mean	0.32	0.29	0.25	0.28	0.29	0.25	0.30	0.26	0.28
Min	0.31	0.22	0.20	0.21	0.20	0.18	0.19	0.19	0.21
Max	0.33	0.43	0.38	0.47	0.39	0.37	0.52	0.38	0.39
h²l	0.02	0.17	0.15	0.32	0.20	0.28	0.37	0.20	0.21

The very low heritability of Rsh72a was probably due to a measurement problem. Apart from that, Rsh data appeared rather stable along time.

Figure 21 presents the evolution of heritabilities for the traits of latex diagnostic. The heritabilities of Drc, Suc and Pi followed a decreasing trend, however less important for Pi.

Figure 21 : Heritabilities of LD traits (9 Latex Diagnostic series from LD72a to LD96).



3.2.4.2. Correlations

Dry rubber content

Table 26 shows positive correlations between the 9 series of Drc BLUPs. However, the levels of the coeficients of correlation between Drc93 and the 8 other traits was lower. Drc traits were also slightly and positively correlated with the girth G7.

Table 26 : Correlations between the 9 series of Drc BLUPs (Dry Rubber Content). The trait G7 was added (girth before tapping, measured in month 59, May 2007). Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Drc72a	Drc72b	Drc74	Drc81	Drc82	Drc92	Drc93	Drc95	Drc96	G7
Drc72a	**1**	0.76	0.63	0.50	0.58	0.45	0.16	0.45	0.32	0.22
Drc72b	0.76	**1**	0.72	0.54	0.69	0.52	0.25	0.43	0.33	0.26
Drc74	0.63	0.72	**1**	0.62	0.62	0.55	0.29	0.47	0.37	0.30
Drc81	0.50	0.54	0.62	**1**	0.47	0.46	0.24	0.37	0.30	0.26
Drc82	0.58	0.69	0.62	0.47	**1**	0.57	0.30	0.45	0.37	0.31
Drc92	0.45	0.52	0.55	0.46	0.57	**1**	0.41	0.38	0.38	0.26
Drc93	0.16	0.25	0.29	0.24	0.30	0.41	**1**	0.24	0.31	0.26
Drc95	0.45	0.43	0.47	0.37	0.45	0.38	0.24	**1**	0.42	0.26
Drc96	0.32	0.33	0.37	0.30	0.37	0.38	0.31	0.42	**1**	0.25

As shown in table 27, in most cases the Drc traits from Drc72a to Drc92 were negatively correlated with all the latex production traits excepted Lp93 and Lp94. This negative correlation was also weaker with Lp92, Lp95, and Lp96. But for the traits Drc93, Drc95, and Drc96, there was in most cases no significant correlation with the latex production traits, probably due to the reduction in Drc variability during the intensive period with stimulations.

Table 27 : Correlations between Drc (Dry Rubber Content) and latex production traits (log-transformed data Lp). Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Drc72a	Drc72b	Drc74	Drc81	Drc82	Drc92	Drc93	Drc95	Drc96
Lp71	-0.30	-0.34	-0.34	-0.30	-0.26	-0.30	ns	ns	ns
Lp72	-0.40	-0.38	-0.30	-0.20	-0.32	-0.26	ns	ns	ns
Lp73	-0.37	-0.38	-0.36	-0.26	-0.32	-0.28	ns	ns	ns
Lp74	-0.43	-0.42	-0.43	-0.34	-0.36	-0.31	ns	-0.16	ns
Lp75	-0.35	-0.34	-0.33	-0.25	-0.30	-0.25	ns	ns	ns
Lp81	-0.44	-0.42	-0.43	-0.52	-0.33	-0.31	ns	-0.19	ns
Lp82	-0.44	-0.45	-0.37	-0.32	-0.50	-0.22	ns	-0.16	ns
Lp83	-0.36	-0.39	-0.31	-0.26	-0.37	-0.23	ns	ns	ns
Lp91	-0.35	-0.35	-0.35	-0.36	-0.29	-0.42	ns	ns	ns
Lp92	-0.20	-0.23	ns	ns	-0.24	-0.25	ns	ns	ns
Lp93	ns	ns	ns	ns	ns	0.15	ns	0.17	0.20
Lp94	ns	ns	ns	ns	ns	ns	ns	ns	ns
Lp95	-0.20	-0.24	-0.18	-0.18	-0.29	-0.17	ns	ns	-0.15
Lp96	-0.21	-0.23	-0.21	ns	-0.30	-0.14	ns	ns	ns

Sucrose content

As shown in table 28, positive correlations were found between sucrose contents with r varying from 0.18 to 0.63 (average r = 0.36).

Table 28 : Correlations between sucrose content (BLUPs, root transformed data RSuc). Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	RSuc72a	RSuc72b	RSuc74	RSuc81	RSuc82	RSuc92	RSuc93	RSuc95	RSuc96
RSuc72a	1	0.63	0.53	0.32	0.25	0.42	0.29	0.24	0.31
RSuc72b	0.63	1	0.53	0.41	0.34	0.38	0.25	0.18	0.20
RSuc74	0.53	0.53	1	0.42	0.40	0.53	0.35	0.27	0.25
RSuc81	0.32	0.41	0.42	1	0.29	0.40	0.28	0.27	0.28
RSuc82	0.25	0.34	0.40	0.29	1	0.42	0.30	0.28	0.23
RSuc92	0.42	0.38	0.53	0.40	0.42	1	0.59	0.39	0.41
RSuc93	0.29	0.25	0.35	0.28	0.30	0.59	1	0.45	0.42
RSuc95	0.24	0.18	0.27	0.27	0.28	0.39	0.45	1	0.45
RSuc96	0.31	0.20	0.25	0.28	0.23	0.41	0.42	0.45	1

As shown in table 29, there were negative correlations between sucrose traits from RSuc72a to RSuc92 and latex production traits from Lp71 to Lp92, with coefficients of correlation varying from r = -0.39 to r = -0.15. This negative correlation was weaker or non significant for production traits from LP93 to LP96. It was also weaker or non significant for sucrose traits from RSuc93 to RSuc96.

Table 29 : Correlations between Sucrose content (root transformed data RSuc) and latex production traits (log-transformed data Lp non-adjusted to the girth). Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	RSuc72a	RSuc72b	RSuc74	RSuc81	RSuc82	RSuc92	RSuc93	RSuc95	RSuc96
Lp71	-0.35	-0.34	-0.32	-0.38	-0.23	-0.35	-0.15	ns	-0.17
Lp72	-0.29	-0.35	-0.32	-0.37	-0.29	-0.35	-0.18	ns	-0.16
Lp73	-0.32	-0.34	-0.29	-0.37	-0.24	-0.31	ns	ns	ns
Lp74	-0.35	-0.35	-0.33	-0.37	-0.31	-0.38	-0.15	ns	ns
Lp75	-0.33	-0.35	-0.29	-0.37	-0.28	-0.35	-0.17	ns	-0.16
Lp81	-0.38	-0.33	-0.39	-0.37	-0.27	-0.39	-0.16	ns	-0.20
Lp82	-0.31	-0.28	-0.29	-0.27	-0.28	-0.38	-0.15	ns	-0.15
Lp83	-0.31	-0.30	-0.31	-0.35	-0.30	-0.37	-0.17	ns	-0.19
Lp91	-0.37	-0.35	-0.36	-0.38	-0.24	-0.37	-0.17	ns	ns
Lp92	-0.22	-0.25	-0.22	-0.34	-0.26	-0.29	-0.23	ns	-0.16
Lp93	Ns	ns	ns	-0.23	ns	ns	-0.16	ns	-0.14
Lp94	-0.18	-0.14	ns	-0.22	-0.16	-0.19	-0.17	ns	ns
Lp95	Ns	ns	ns	-0.15	-0.18	-0.19	ns	ns	ns
Lp96	Ns	-0.18	ns	-0.15	-0.23	-0.21	ns	ns	ns

The three traits Suc72a, Suc72b, and Suc74 measured in 2007 showed the highest mean levels of sucrose content combined with rather high heritabilities.

Inorganic phosphorus content

As shown in table 30, positive correlations were found between inorganic phosphorus traits (in average r = 0.57).

Table 30 : Correlations between Inorganic Phosphorus content. Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Pi72a	Pi72b	Pi74	Pi81	Pi82	Pi92	Pi93	Pi95	Pi96
Pi72a	1.00	0.83	0.60	0.64	0.68	0.66	0.48	0.46	0.49
Pi72b	0.83	1.00	0.61	0.64	0.71	0.67	0.54	0.49	0.49
Pi74	0.60	0.61	1.00	0.53	0.54	0.55	0.38	0.38	0.34
Pi81	0.64	0.64	0.53	1.00	0.66	0.54	0.35	0.39	0.40
Pi82	0.68	0.71	0.54	0.66	1.00	0.71	0.67	0.55	0.64
Pi92	0.66	0.67	0.55	0.54	0.71	1.00	0.70	0.60	0.65
Pi93	0.48	0.54	0.38	0.35	0.67	0.70	1.00	0.66	0.66
Pi95	0.46	0.49	0.38	0.39	0.55	0.60	0.66	1.00	0.67
Pi96	0.49	0.49	0.34	0.40	0.64	0.65	0.66	0.67	1.00

As shown in table 31, positive correlations were found between inorganic phosphorus (Pi) and latex production (in average r = 0.42). However the coefficients of correlation were lower for latex production traits from Lp92 to LP96.

Table 31 : Correlations between inorganic phosphorus (log-transformed data for LPi81) and production traits (log-transformed data Lp). Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Pi72a	Pi72b	Pi74	LPi81	Pi82	Pi92	Pi93	Pi95	Pi96
Lp71	0.63	0.55	0.43	0.51	0.53	0.52	0.40	0.39	0.43
Lp72	0.68	0.57	0.42	0.41	0.52	0.51	0.39	0.36	0.43
Lp73	0.62	0.60	0.47	0.48	0.57	0.56	0.43	0.38	0.46
Lp74	0.63	0.60	0.50	0.49	0.56	0.51	0.38	0.36	0.46
Lp75	0.53	0.47	0.42	0.43	0.50	0.51	0.32	0.35	0.39
Lp81	0.63	0.58	0.45	0.70	0.53	0.51	0.36	0.36	0.40
Lp82	0.59	0.53	0.40	0.54	0.62	0.51	0.40	0.37	0.50
Lp83	0.57	0.51	0.38	0.40	0.55	0.54	0.43	0.38	0.45
Lp91	0.57	0.50	0.39	0.55	0.54	0.61	0.35	0.44	0.47
Lp92	0.43	0.37	0.26	0.26	0.42	0.53	0.37	0.38	0.48
Lp93	0.22	0.11	0.07	0.12	0.22	0.22	0.25	0.28	0.37
Lp94	0.27	0.22	0.20	0.23	0.28	0.31	0.27	0.37	0.44
Lp95	0.40	0.34	0.24	0.30	0.35	0.29	0.25	0.40	0.45
Lp96	0.41	0.32	0.21	0.26	0.38	0.35	0.27	0.29	0.48

Thiol content

As shown in table 32, positive correlations were found between thiol content traits (in average r = 0.41).

Table 32 : Correlations between thiol content traits. Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Rsh72a	Rsh72b	Rsh74	Rsh81	Rsh82	Rsh92	Rsh93	Rsh95	Rsh96
Rsh72a	1.00	0.43	0.32	0.28	0.21	0.36	0.24	0.25	0.21
Rsh72b	0.43	1.00	0.49	0.45	0.32	0.46	0.35	0.40	0.31
Rsh74	0.32	0.49	1.00	0.46	0.36	0.43	0.33	0.44	0.36
Rsh81	0.28	0.45	0.46	1.00	0.43	0.54	0.41	0.41	0.35
Rsh82	0.21	0.32	0.36	0.43	1.00	0.51	0.54	0.42	0.36
Rsh92	0.36	0.46	0.43	0.54	0.51	1.00	0.62	0.52	0.50
Rsh93	0.24	0.35	0.33	0.41	0.54	0.62	1.00	0.58	0.50
Rsh95	0.25	0.40	0.44	0.41	0.42	0.52	0.58	1.00	0.50
Rsh96	0.21	0.31	0.36	0.35	0.36	0.50	0.50	0.50	1.00

In most cases, no correlation was found between thiol content and rubber production.

Correlations between the different traits of latex diagnostic

In most cases, there were positive but moderate correlations between Drc and Suc for the LD series from LD72 to LD92. However, in most cases, correlations of Drc with Suc traits from RSuc93 to RSuc96, and correlations of Suc with Drc traits from Drc93 to Drc96 were non significant (table 33).

Table 33 : Correlations between Drc and RSuc traits. Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Drc72a	Drc72b	Drc74	Drc81	Drc82	Drc92	Drc93	Drc95	Drc96
RSuc72a	0.2	0.22	0.2	0.18	0.15	0.14	ns	ns	ns
RSuc72b	0.18	0.24	0.16	ns	0.17	0.18	ns	ns	ns
RSuc74	0.33	0.31	0.25	0.24	0.26	0.31	0.14	0.23	ns
RSuc81	0.15	ns	ns	ns	ns	0.14	ns	ns	ns
RSuc82	0.36	0.28	0.14	ns	0.21	0.18	ns	0.16	ns
RSuc92	0.25	0.32	0.18	0.17	0.33	0.26	0.15	0.23	ns
RSuc93	ns	ns	ns	ns	ns	ns	0.14	ns	ns
RSuc95	ns	ns	ns	ns	ns	ns	ns	ns	ns
RSuc96	ns	Ns	ns	ns	ns	ns	ns	ns	ns

In all cases there were negative correlations between Drc and Pi for traits from Drc72a to DRc92, and from Pi72a to Pi92 (table 34).

Table 34 : Correlations between dry rubber content and inorganic phosphorus traits. Df = 194 ; threshold r = 0.14 for α = 0.05.

Traits	Drc72a	Drc72b	Drc74	Drc81	Drc82	Drc92	Drc93	Drc95	Drc96
Pi72a	-0.44	-0.51	-0.49	-0.36	-0.48	-0.44	-0.15	-0.30	-0.23
Pi72b	-0.47	-0.52	-0.49	-0.39	-0.47	-0.48	-0.16	-0.37	-0.25
Pi74	-0.38	-0.39	-0.42	-0.36	-0.35	-0.37	ns	-0.22	-0.17
Pi81	-0.42	-0.47	-0.53	-0.63	-0.43	-0.41	ns	-0.29	-0.20
Pi82	-0.34	-0.42	-0.41	-0.31	-0.49	-0.34	ns	-0.29	-0.17
Pi92	-0.25	-0.29	-0.32	-0.24	-0.32	-0.48	-0.19	-0.16	ns
Pi93	ns	ns	ns	ns	-0.17	ns	ns	ns	ns
Pi95	ns	ns	ns	ns	-0.14	-0.25	ns	ns	ns
Pi96	-0.17	-0.16	ns	ns	-0.19	-0.21	ns	ns	ns

In all cases, for the traits from RSuc72a to RSuc92 and from Pi72a to Pi92, there were negative correlations between Suc and Pi traits (table 35).

Table 35 : Correlations between sucrose content (root-transformed data) and inorganic phosphorus (log-transformed data for LPi81). Df = 194 ; threshold r = 0.14 for α = 0.05.

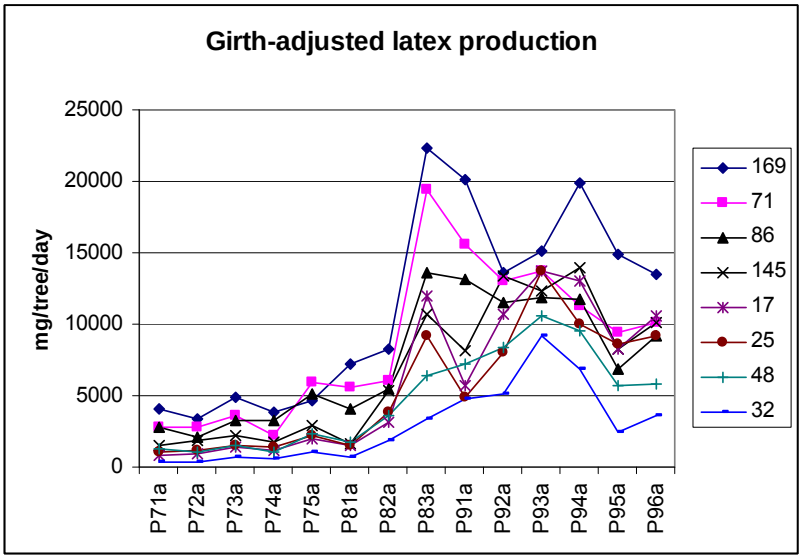
Traits	RSuc72a	RSuc72b	RSuc74	RSuc81	RSuc82	RSuc92	RSuc93	RSuc95	RSuc96
Pi72a	-0.24	-0.35	-0.34	-0.31	-0.26	-0.38	-0.14	ns	ns
Pi72b	-0.25	-0.31	-0.37	-0.28	-0.23	-0.40	Ns	ns	ns
Pi74	-0.32	-0.40	-0.29	-0.27	-0.19	-0.30	Ns	ns	-0.14
Pi81	-0.32	-0.25	-0.36	-0.18	-0.15	-0.34	Ns	ns	ns
Pi82	-0.28	-0.24	-0.35	-0.22	-0.16	-0.43	ns	ns	ns
Pi92	-0.25	-0.27	-0.36	-0.33	-0.20	-0.40	-0.14	ns	ns
Pi93	-0.14	ns	-0.21	-0.16	-0.14	-0.32	ns	ns	ns
Pi95	ns	ns	-0.19	ns	ns	-0.22	ns	ns	ns
Pi96	ns	ns	-0.21	-0.20	-0.18	-0.26	ns	ns	ns

Evolution of the traits with intensification

For showing the joint evolution of production and LD traits, a set of 8 genotypes was chosen (n° 169, 71, 86, 145, 17, 25, 48, and 32 in decreasing order of average latex production), covering the whole range of the girth-adjusted latex productions of the 196 genotypes. Figure 22 shows the evolution of latex production traits. Figure 23 (a, b, c, d, e, f, g, h) shows the evolution of other traits including growth before and during tapping, W1 and plugging index, and the traits of the 9 latex diagnostic series.

Logically, no relationship was observed in figure 23-a between growth before tapping (G59) and girth-adjusted production. A negative relationship was observed in figure 23-b between girth increment during tapping (Gitap) and latex production. In figure 23-c, no relationship was observed between W1 (latex production of the first 5 minutes) and latex production, but a negative relationship was observed between plugging index and latex production in figure 23-d. Negative relationships between Drc and production, and between Suc and production were observed in figures 23-e and 23-f. A positive relationship between Pi and production was observed in figure 23-g. No relationship between Rsh and production was observed in figure 23-h.

Figure 22 : Evolution of girth-adjusted production traits for 8 genotypes covering the range of variation of production (n°169 = highest yielder ; n°32 = lowest yielder).



Figures from 23-c to 23-g show a decrease in variability of W1, PI, Drc, Suc, and Pi during the intensification.

Figure 23 (a, b, c, d, e, f, g, h) : Variability of the traits for a set of 8 genotypes covering the range of variation of latex production, from genotype 169 (highest yielder) to genotype 32 (lowest yielder).

3.2.4.3. Multiple regression

We used the growth and LD traits measured during the first tapping year (2007) as six « independent » variables in a multiple regression analysis for predicting the 14 production traits from Lp71a to Lp96a (« dependent variables ») measured from 2007 to 2009. The independent variables were G59 (girth before tapping) and Gi5964 (girth increment during the tapping period of 2007), and the averages of the three series of LD traits of 2007 (Drc07, Suc07, Pi07, and Rsh07). In fact Rsh73a was not taken into account due to its very low heritability.

Table 36 shows that the percentage of explanation of the productions from Lp71a to Lp91a (R^2 varying from 0.46 to 0.64) was higher than that of the productions from Lp92a to Lp96a (R^2 varying from 0.08 to 0.31). The lowest R^2 was observed for Lp93a.

All the 14 production traits were mainly explained, positively, by Pi07. For Lp93a, only Pi07 provided a significant contribution, but at its lowest level (normalized coefficient = 0.286). Thus the metabolic activity was the most important factor explaining the variability of the genotypes for all the production traits.

The girth before tapping G59 was the second most important factor in the multiple regression, and it provided a positive contribution to the statistical model, excepted for Lp93a, Lp95a, and Lp96a. Gi5964 provided a negative contribution for the production traits of 2007 and for Lp83a. The negative contribution of Drc07 was not significant for Lp92a, Lp93a, and Lp94a. Suc07 was significant only for Lp81a and Lp91a, with a negative contribution. Although the effects were not significant, it must be noticed that the contribution of Suc07 became positive and followed an increasing trend from Lp91a to Lp96a.

Rsh07 brought a significant but negative contribution for the last traits Lp95a and Lp96a, which is the only observation of a physiological distinction of these two production traits from the others.

Table 36 : Multiple regression explaining the girth-adjusted production traits from Lp71a to Lp96a (Xlstat software). Coefficients of determination R^2. « Normalized

coefficients » (or « beta » coefficients) indicating the importance of the contributions (positive or negative) of the independant variables to the explanation of the production traits. In bold : significant normalized coefficients (α = 0.05).

Traits	R^2	G59	Gi5964	Drc07	Suc07	Pi07	Rsh07
Lp71a	0.56	**0.196**	**-0.149**	**-0.129**	-0.095	**0.539**	0.019
Lp72a	0.59	**0.203**	**-0.214**	**-0.164**	-0.046	**0.516**	-0.014
Lp73a	0.59	**0.136**	**-0.169**	**-0.181**	-0.040	**0.536**	-0.012
Lp74a	0.64	**0.171**	**-0.191**	**-0.241**	-0.055	**0.502**	-0.037
Lp75a	0.54	**0.200**	**-0.218**	**-0.212**	-0.076	**0.426**	0.002
Lp81a	0.54	**0.157**	-0.075	**-0.244**	**-0.144**	**0.461**	0.002
Lp82a	0.46	**0.207**	-0.104	**-0.276**	-0.051	**0.415**	-0.050
Lp83a	0.48	**0.200**	**-0.209**	**-0.183**	-0.064	**0.418**	-0.051
Lp91a	0.51	**0.136**	-0.047	**-0.185**	**-0.137**	**0.511**	-0.030
Lp92a	0.31	**0.143**	-0.097	-0.084	-0.044	**0.431**	-0.054
Lp93a	0.08	0.094	-0.058	0.038	0.051	**0.286**	-0.092
Lp94a	0.18	**0.182**	-0.096	-0.045	0.045	**0.355**	-0.112
Lp95a	0.25	0.129	-0.018	**-0.157**	0.101	**0.420**	**-0.151**
Lp96a	0.24	0.074	-0.019	**-0.176**	0.082	**0.386**	**-0.163**

3.2.5. Defoliation earliness

Defoliation earliness (table 37) was scored from S1 (not yet defoliated) to S5 (completely refoliated), at the beginning of each year in 2005 (Def31, month 31 = January 2005), 2006 (Def43), 2007 (Def55), 2008 (Def66), and 2009 (Def79). In 2007, the two scorings were separarated by two weeks. In 2008 and 2009, there were 5 and 4 scorings respectively, each separated from the preceding one by one week. For each date of observation, an average score was calculated for each genotype. Among the genotypes, all the traits were positively correlated between each other, excepted between Def31 and Def79d (no correlation), with the coefficients of correlation varying from r = 0.18 to r = 0.81 (in average, r = 0.47).

Table 37 : General data about defoliation scorings. Number of trees per trait and per level of scoring, from S1 to S5. Total number of trees scored and mean score per date.

Traits	Year	S1	S2	S3	S4	S5	Nb trees	Mean score
Def31	2005	169	877	285	410	556	2297	3.134
Def43	2006	812	625	709	273	98	2517	2.293
Def55a	2007	122	1472	448	427	20	2489	2.498
Def55b	2007	18	82	84	1261	1059	2504	4.302
Def66a	2008	783	1671	60	0	0	2514	1.712
Def66b	2008	30	2172	307	5	0	2514	2.114
Def66c	2008	0	1634	840	41	0	2525	2.367
Def66d	2008	0	780	1260	473	0	2513	2.878
Def66e	2008	0	162	300	1761	290	2513	3.867
Def79a	2009	95	2016	389	16	0	2516	2.130
Def79b	2009	17	1198	972	321	5	2513	2.641
Def79c	2009	0	553	572	1338	51	2514	3.353
Def79d	2009	0	37	92	1138	1247	2514	4.430

3.2.6. Leaves dimensions

Two series of measurements were carried out in 2006 at 48 months and 52 months (table 38). For each series, one leaf per tree was collected on 2308 trees. For each leaf, measurements were carried out for the fresh weight (FW1, FW2), the length (L1, L2) and the width (W1, W2) of each of the three leaflets. The total leaf area of the three leaflets (LA2) was measured only for the second series. The means of the three leaflets were also calculated for fresh weight (FW1, FW2), length (L1, L2), and width (W1, W2).

Table 38 : Mean values of the measured traits. FW in grammes, L and W in cm, LA in cm².

	First series month 48			Second series month 52			
Trait	FW11	FW12	FW13	FW21	FW22	FW23	LA2
Mean	1.12	1.26	1.11	0.93	1.12	0.94	63.07
Trait	L11	L12	L13	L21	L22	L23	
Mean	15.47	16.85	15.32	13.54	15.45	13.56	
Trait	W11	W12	W13	W21	W22	W23	
Mean	6.45	6.55	6.44	6.01	6.31	6.00	

Heritabilities of leaves dimensions traits were low. For the first series, h²l varied from 0.09 to 0.13. For the second series, h²l varied from 0.05 to 0.10.

There were significant positive correlations between all the traits relative to leaves dimensions and the girth measured at 47 months (G47), at the time when the leaves were collected and measured (r varying from 0.14 to 0.38 ; df = 194 ; threshold r = 0.14 for α = 0.05).

3.2.7. Bark thickness

Bark thickness was first measured in April 2008 on the four sides of each tree (B1, B2p, B3, and B4. Then two repeated measurements were made on the tapping panel side in June 2009 (B5p and B6p). Heritabilities of bark thickness traits, presented in table 39, were moderate. Heritability was higher for the mean of the six measurements (h^2I = 0.31 for the non-adjusted Bm).

Table 39 : Heritabilities and means of non-adjusted and girth-adjusted bark depth traits.

Non adjust.	B1	B2p	B3	B4	B5p	B6p	Bm
h^2I	0.12	0.14	0.18	0.18	0.25	0.24	0.31
Girth-adjusted	B1a	B2pa	B3a	B4a	B5pa	B6pa	Bma
h^2I	0.08	0.12	0.16	0.15	0.23	0.22	0.30
Means (mm)	5.435	6.375	6.412	6.437	7.071	6.798	6.421

The six bark depth traits were positively correlated with the girth of the trees before tapping (G59), with r varying from 0.45 to 0.58 (table 40). For this reason, the bark depth traits were also analysed by adjusting them to the girth G59 (covariance analysis). The traits B2p, B3, and B4 were negatively correlated with the cumulated latex production of the two years 2007 and 2008 (Lpcum78, log-transformed data).

Table 40 : Coefficients of correlation between non-adjusted bark depth traits (df = 194 ; threshold r = 0.14 for α = 0.05).

Variables	G59	B1	B2p	B3	B4	B5p	B6p
G59	**1**	0.58	0.45	0.45	0.47	0.58	0.53
B1	0.58	**1**	0.62	0.63	0.60	0.68	0.63
B2p	0.45	0.62	**1**	0.89	0.85	0.70	0.61
B3	0.45	0.63	0.89	**1**	0.86	0.69	0.62
B4	0.47	0.60	0.85	0.86	**1**	0.67	0.57
B5p	0.58	0.68	0.70	0.69	0.67	**1**	0.90
B6p	0.53	0.63	0.61	0.62	0.57	0.90	**1**
Lpcum78	0.14	0.01	-0.22	-0.28	-0.23	-0.11	-0.05

3.2.8. Die-back

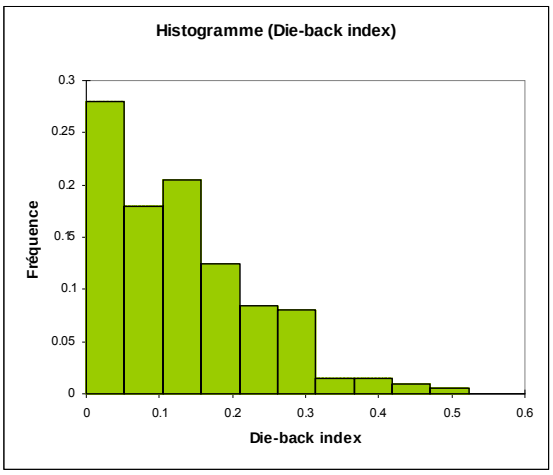
The die-back phenomenon (table 41) was scored from 1 (dead trees) to 5 (healthy trees with no visible symptom). The numbers of trees for each genotype and each score were used for building a contingency table. A χ^2 test showed that this scoring indicated significant differences between the genotypes, and therefore a genetic effect. The coefficients and the numbers of trees per score and per genotype were used for calculting a weighed mean for each genotype (die-back index).

Table 41 : Status of the trees for the die-back symptoms. Scoring from 1 to 5.

Score	Coefficient	Symptoms	Nb trees	%
1	1.00	Dead	176	6.5
2	0.75	Not completely dead	143	5.3
3	0.50	Dead branches	91	3.3
4	0.20	Yellow leaves	82	3.0
5	0.00	Healthy	2227	81.9
Total	-	-	2719	100

Figure 24 shows the distribution of the values of the die-back index for all the genotypes. This index varied among the 196 genotypes from 0.000 (resistant) to 0.513 (very susceptible). This distribution was very dissymetric : only 43 susceptible genotypes had a die-back index higher than 0.200.

Figure 24 : Distribution of the values of die-back index of the 196 genotypes.



3.2.9. Molar mass distributions

3.2.9.1. Heritabilities

The statistical model used for analysing the traits issued from SEC was different from that used for the other traits. Heritabilities of the traits, as shown in table 42, were medium or rather high, varying from 0.19 to 0.46.

Table 42 : Heritabilities of the traits related with the macromolecular structure of native rubber.

Trait	h²I
M_{z+1}	0.19
M_z	0.26
M_w	0.42
M_n	0.37
Ip	0.39
R21	0.46
Gel	0.31

3.2.9.2. Correlations

The girth G59 and the girth-adjusted cumulated production of 2007 and 2008 (Lpcum0708a) from all the trees of the trial were introduced in the correlation study. Table 43 shows the correlations between G59, Lpcum0708a, and the traits related with the macromolecular structure of native rubber.

G59 was positively correlated with M_w and M_n, but negatively correlated with I_p and R21. Lpcum0708a was positively correlated with M_z and I_p but negatively correlated with M_n and Gel. The four molar mass traits M_{z+1}, M_z, M_w, and M_n were positively correlated between each other, but negatively correlated with R21. M_w and M_n were positively correlated with Gel, but negatively correlated with I_p.

Table 43 : Correlations between girth before tapping (G59), cumulated production of 2007 and 2008 (LPcum0708a), and the traits related with the macromolecular structure of native rubber. Df = 194 ; threshold r = 0.14 for α = 0.05.

Variables	G59	Lpcum0708a	M_{z+1}	M_z	M_w	M_n	Ip	R21	Gel
G59	1.00	ns	ns	ns	0.19	0.24	-0.21	-0.18	ns
Lpcum0708a	ns	1.00	ns	0.17	ns	-0.21	0.34	ns	-0.29
M_{z+1}	ns	ns	1.00	0.92	0.65	0.30	ns	-0.44	-0.16
M_z	ns	0.17	0.92	1.00	0.85	0.42	ns	-0.69	ns
M_w	0.19	ns	0.65	0.85	1.00	0.73	-0.32	-0.94	0.26
M_n	0.24	-0.21	0.30	0.42	0.73	1.00	-0.85	-0.75	0.52
Ip	-0.21	0.34	ns	ns	-0.32	-0.85	1.00	0.39	-0.53
R21	-0.18	ns	-0.44	-0.69	-0.94	-0.75	0.39	1.00	-0.41
Gel	ns	-0.29	-0.16	ns	0.26	0.52	-0.53	-0.41	1.00

3.3. QTL mapping

Loci positions were hereafter indicated by a code number for the linkage group or chromosome (from 1 to 18) and by the position on the chromosome (in centimorgans). For example, g3-60 is the indication of the locus on chromosome n° 3 at the position of 60 cM. The QTLs, which were often detected repeatedly for many traits at slightly different positions, were named after their most probable position characterized by a high LOD score and a high percentage of explained phenotypic variance (%exp). A LOD score threshold of 4.5 was used as significance level for all the studied traits. For each QTL, the average levels of the four genotypic classes « ac », « ad », « bc », and « bd » were presented by indexes relative to the level of class « ac ». Therefore, all the indexes of the class « ac » were equal to 100. This makes easier the reading of the different effects of allelic substitution in the classes « ad », « bc », and « bd » by reference to the « standard » effect of the class « ac ».

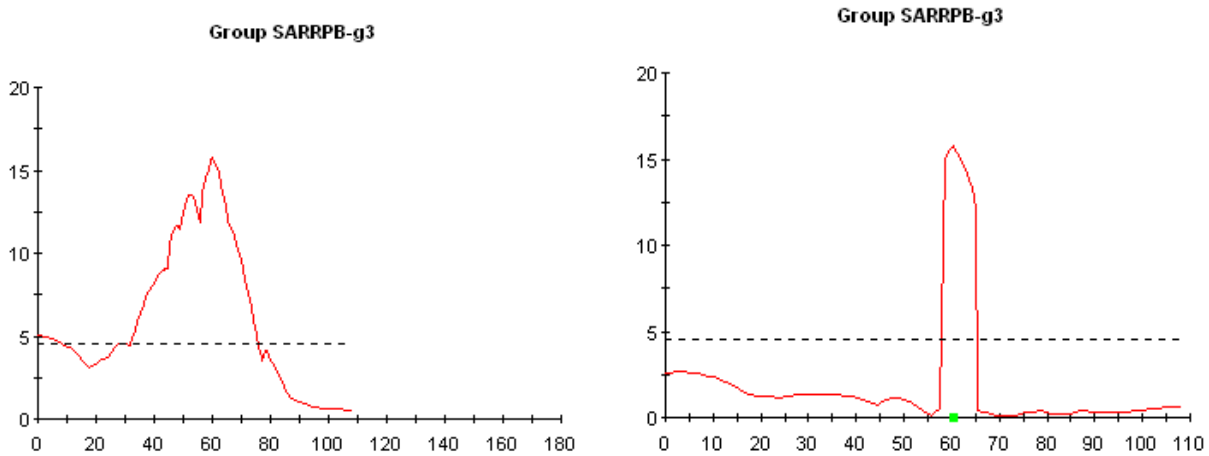
3.3.1. Growth before tapping (2002-2007)

The main result of QTL mapping for growth was the detection of the major QTL g3-60. We hereafter present first the results about this QTL, and then the results about the other detected QTLs.

3.3.1.1. QTL g3-60

Figure 25 shows the curves of LOD score for the detection of the QTL g3-60 with its maximum effect in the case of the trait G59, by Interval Mapping method (IM) and by MQM method after putting the marker a312 as cofactor in the analysis. The LOD support interval observed in the IM method, at 1 LOD unit below the peak, was from 58.1 to 62.4 cM. Therefore there would be a « confidence interval » of less than 5 cM for the position of this QTL. The MQM method showed that there was apparently only one QTL for this trait on this chromosome, and in most cases, the most probable position was g3-60, which is exactly the position of the marker a312.

Figure 25 : LOD scores observed from the analysis of the girth trait G59 by Interval Mapping (left) and MQM (right) methods. In the MQM method, the market a312, positionned at locus g3-60 was used as cofactor. Significance threshold LOD = 4.5 (dashed horizontal line).



The characteristics of the QTL g3-60, detected for height and girth traits are presented in table 44. The QTL g3-60 was detected for the girth traits (Ga or G) from month 18 to 59 (from Ga18 or G18 to G59). At its maximum effect, for G59

which represented the cumulative effect of growth over 5 years, the QTL explained 31 % of the phenotypic variance (the variance between the BLUPs of the 196 progenies). The QTL was detected also for the girth increment Gai1218 in the season R2, just before the beginning of the drought, and for the last three girth increments Gi4347, Gi4753, and Gi5359 (seasons D4, R5, and D5 after the drought). In all these cases, the genotypic class « bc » was the lowest of the four classes. The very low relative level of « bc » for Gi4347 during a period of slow growth in the dry season D4, must be related with the fact that the absolute mean value of Gi4347 was very low, and its coefficient of variation very high.

The QTL g3-60 appeared to be predominantly associated with the growth in girth. However it was also detected for H53 with the class « bc » being the lowest, at a time when its effect was maximum for the girth. More astonishingly, the QTL was also detected for Hi1723 with a LOD score a little below the significance threshold, but in this case with « bc » being the highest.

The biomass traits issued from the conversion of girth traits were also submitted to QTL detection ; they provided results very similar to those which were obtained with girth, with only one change : the QTL g3-60 was found significant for the biomass increment Biom2331 in the rainy season R3.

Table 44 : Characteristics of the QTL g3-60 for growth traits before tapping. Positions varying from 59 to 68 cM. Index 100 for the genotypic class « ac » taken as reference. LOD was not much below below significance level for Hi1723.

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
Hi1723	3	56	4.3	10	100	97	121	99
H53	3	59	6.0	14	100	100	97	98
Ga18	3	60	9.1	18	100	100	95	98
G18	3	60	6.9	15	100	100	95	99
Ga23	3	60	9.5	20	100	100	95	99
G23	3	60	7.3	16	100	100	96	99
G31	3	60	8.0	17	100	100	97	99
G36	3	60	6.5	14	100	100	98	99
G43	3	60	9.1	19	100	100	98	99
G47	3	60	12.4	26	100	100	97	99
G53	3	60	13.4	27	100	100	96	99
G59	3	60	15.8	31	100	101	96	99
Gai1218	3	60	9.4	18	100	102	92	98
Gi4347	3	59	5.3	12	100	97	51	85
Gi4753	3	60	8.4	18	100	100	93	98
Gi5359	3	60	9.7	18	100	104	88	97
Biom2331	3	50	7.2	16	100	99	93	96

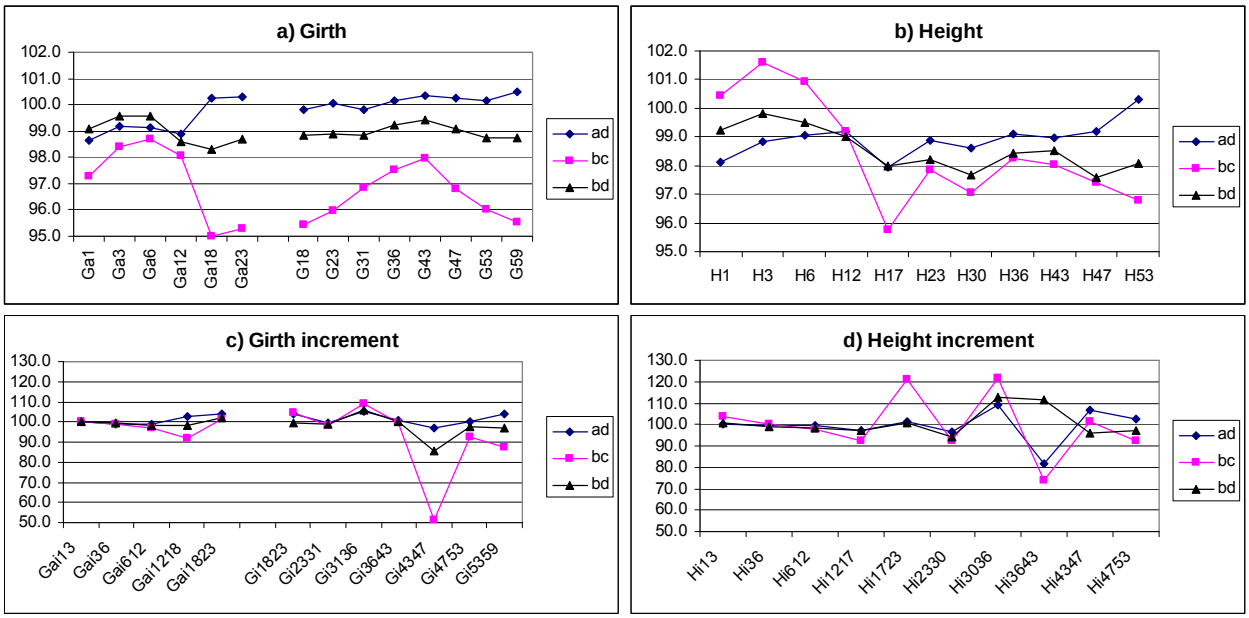
For better understanding these observations, we examined the evolution of the four genotypic classes of the locus g3-60, even in the cases where the QTL was non-significant (figure 26).

For girth, the curves are discontinuous because there was a change in the height of measurement (measurements at 10-cm high from month 1 to 23, and at 1.0 and 1.7-m high from month 18 to 59). As soon as from month 1, the class « bc » was lower than the three other classes, and the difference became still much lower during the fast-growing rainy season R2 (from 12 to 18 months). Then the difference between « bc » and the other classes was reduced along the whole drought period, but it increased again after the drought.

For height, the class « bc » was higher than the other classes during the first semester after planting. Afterwards, the curve of « bc » for height roughly followed the evolution of the curve for girth.

Considering the increments during the seasons D2 and D3, the class « bc » seemed higher than the other classes for girth and more importantly for height, and the QTL g3-60 was almost significant for the increment Hi1723 during D2 (LOD score = 4.3)

Figure 26 : Evolution of the average value indexes of the four genotypic classes of the locus g3-60. Value index 100 for the class « ac » not figured.



The values of G59 for the four genotypic classes of the QTL g3-60, for RRIM600 and for the highest and the lowest genotypes (n° 83 and n° 1 respectively) are shown in table 45. The allelic classes of the nearest marker (a312) are also shown. Between the two parents, the allelic recombination of the QTL was « ab x cd » corresponding to the recombination « fe x eg » of the marker a312 (the opposed phase of RRIM600 was taken into account for a312). Therefore both parents had the allele « e » at the marker. For RRIM600, this allele corresponded to the allele « b » of the QTL, and for PB217, this allele corresponded to the allele « c » of the QTL. However, the alleles « b » and « c » of the QTL may be different.

Table 45 : Values of G59 (cm) for the genotypic classes of the QTL g3-60, for RRIM600 and genotypes n° 83 and n° 1. Allelic classes of the nearest marker (a312).

QTL g3-60	Nb	Marker a312	G59
ac	48	ef	31.77
ad	50	fg	31.99
bc	40	ee	30.40
bd	57	eg	31.38
RRIM600	1	ef	30.84
n° 83 (max)	1	fg	34.35
n° 1 (min)	1	ee	29.28

3.3.1.2. Other QTLs detected for growth traits

Table 46 shows the characteristics of the QTLs other than g3-60 and detected for growth traits before tapping. A total of 10 QTLs (including g3-60) were detected for growth before tapping. The QTL g5-16 was specifically detected for the trait Hi1723 measured during the very dry season D2. Similarly, the QTL g17-84 was specific of the trait Hi3643 which showed a very low increment during the season R4, after the drought. The two positions g12-20 and g12-32 were distinguished as two different QTLs due to their different effects on the genoytpic class « bd ». Concerning the biomass increment Biom5359, the QTL g11-102, which was not significant for Gi5359, was detected.

Table 46 : Characteristics of the QTLs other than g3-60, detected for growth before tapping. Results presented in the order of the chromosome numbers and positions. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
Hi1723	5	16	4.9	11	100	91	113	88
H47	7	105	5.2	11	100	103	102	103
H30	8	84	5.7	13	100	101	102	104
Hi2330	10	100	5.2	11	100	103	111	115
Biom5359	11	102	4.6	7	100	94	103	94
Gai1218	12	20	5.6	12	100	100	101	93
Gi5359	12	32	7.7	16	100	102	107	114
Biom5359	12	33	6.5	11	100	101	107	114
Gai1218	16	7	4.6	7	100	103	96	100
Hi3643	17	84	4.7	12	100	303	-13	58

3.3.1.3. QTLs detected for growth during tapping

Tapping was carried out in 2007 from month 59 to 64, in 2008 from month 71 to 76, and in 2009 from month 82 to 89. The distinction between growth before tapping and growth during tapping is due to the well-known effect of tapping and latex production on the reduction of girth increments.

Since the measurement of H53, the trees were higher than 8 meter, which made the height measurements more difficult. Moreover the canopy was closed and practically, height measurements were possible only in the dry seasons during the defoliation period. Therefore, height was measured only twice, in months 67 (January 2008) and 79 (January 2009).

Table 47 presents the 6 QTLs detected for growth during tapping, from month 59 to 89. For height traits, the QTL g8-89 (LOD = 4.0, below the significance threshold) was detected for H67, and the QTL g9-26 was detected for H79. For girth traits, four QTLs were detected, including the major QTL g3-60, and the three other QTLs g5-22, g16-9, and g18-59.

The QTL g3-60 was detected for the seven traits of girth during tapping from G64 to G89, for the girth increment Gi5964 (rainy season R6, during tapping in 2007), and for the girth increments Gi8285 and Gi8589 (rainy season R8, during tapping in 2009). Its maximum effect was observed for G79 with LOD = 21.6 and %exp = 31 %.

The QTL g16-9 was also detected for the seven girth traits, and for the girth increments Gi5964 and Gi71-76 (rainy seasons R6 and R7, during tapping in 2007 and 2008). The effect of this QTL was rather important, with its maximum for Gi5964 with LOD = 17.0 and %exp = 31 %. In fact, it will be shown later on that this QTL was a major QTL associated with latex production. Its effect on girth was here directly related with the general negative correlation between production and girth increment during tapping.

The two other QTLs, g5-22 and g18-59, although repeatedly observed on many girth traits, had small effects with LOD scores lower than 6.0 and %exp lower than 10 %.

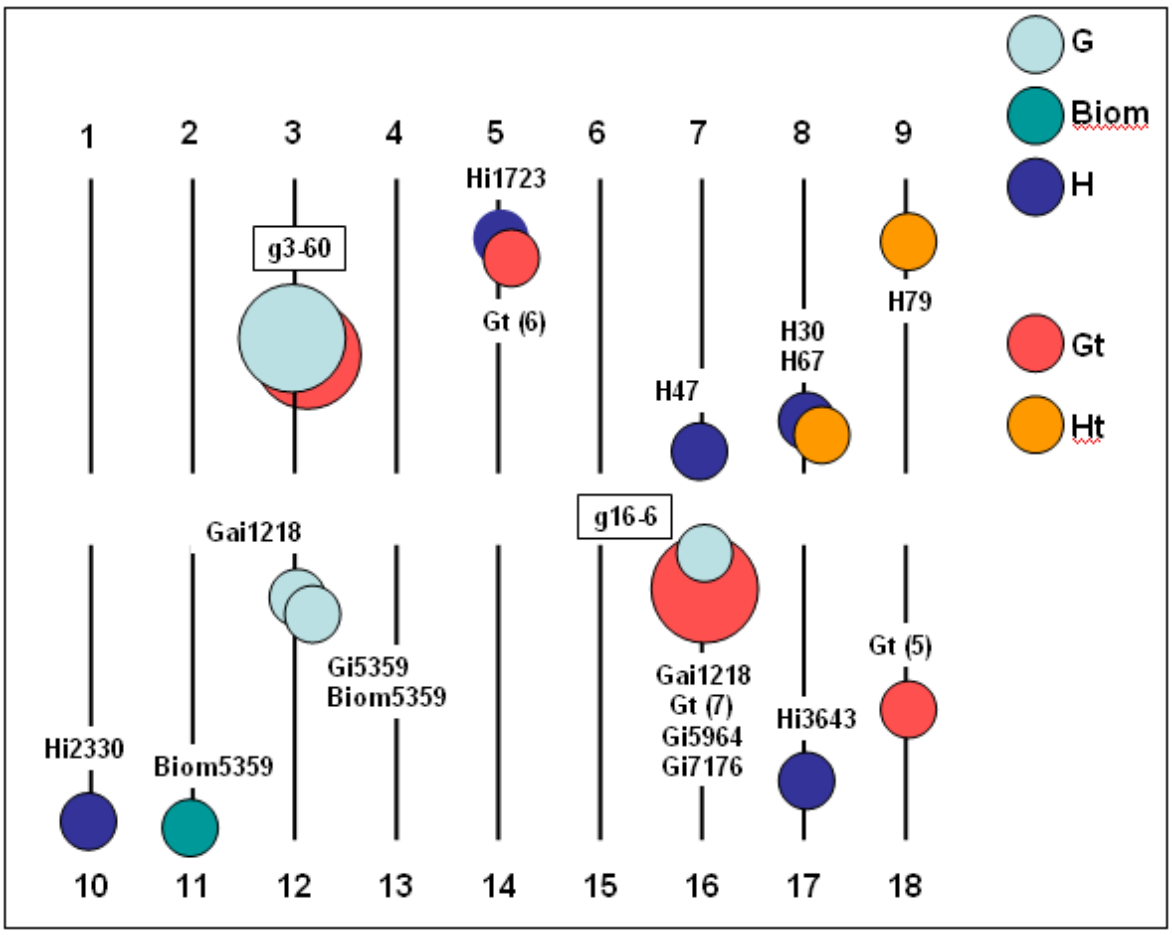
Table 47 : Characteristics of the QTLs detected for growth traits measured after the beginning of tapping. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	Bc	bd
G64	3	60	16.0	25	100	100	94	99
G71	3	60	17.4	28	100	100	94	99
G76	3	60	19.5	27	100	100	93	98
G79	3	61	21.6	31	100	100	93	98
G82	3	60	21.6	30	100	101	93	98
G85	3	60	21.7	30	100	100	92	98
G89	3	60	21.2	30	100	100	92	98
Gi5964	3	60	5.8	9	100	98	86	98
Gi8285	3	60	7.3	16	100	99	73	92
Gi8289	3	61	7.9	17	100	96	77	93
G64	5	22	4.5	6	100	99	102	99
G76	5	22	5.5	7	100	98	102	99
G79	5	22	4.7	6	100	99	102	98
G82	5	23	4.8	6	100	99	102	98
G85	5	22	4.6	6	100	99	102	99
G89	5	22	4.6	6	100	99	102	98
G64	16	9	12.0	19	100	103	97	101
G71	16	9	10.0	15	100	102	97	101
G76	16	8	12.6	16	100	103	96	100
G79	16	8	11.6	15	100	102	97	100
G82	16	8	11.4	15	100	102	97	100
G85	16	8	10.6	13	100	102	96	100
G89	16	9	9.8	12	100	102	96	100
Gi5964	16	7	17.0	31	100	114	86	100
Gi7176	16	3	9.1	20	100	110	89	100
G76	18	59	4.5	5	100	102	102	103
G79	18	57	5.0	6	100	102	102	103
G82	18	59	5.0	6	100	102	102	103
G85	18	59	5.1	6	100	102	103	103
G89	18	57	5.6	6	100	103	103	103
Gi8289	18	60	4.6	9	100	108	118	114
H67	8	89	4.0	10	100	101	102	103
H79	9	26	6.0	17	100	100	103	101

3.3.1.4. Summary of the QTLs detected for growth

Figure 27 recalls the positions of the 13 QTLs detected for growth before tapping (10 QTLs) and during tapping (6 QTLs). Whereas the major QTL g3-60 was detected before and during tapping, the major QTL g16-6 was detected quite exclusively during tapping, in relation with rubber production (with the exception of the trait Gai1218).

Figure 27 : Locations of the 13 QTLs detected for growth before tapping and during tapping. The large circles refer to the two major QTLs g3-60 and g16-6, which were detected repeatedly. G, Biom, and H indicate traits of girth, biomass, or height before tapping. Gt and Ht indicate traits of girth and height during tapping. Gt(5, 6, 7) indicates that 5, 6, or 7 traits of girth during tapping were detected repeatedly.



3.3.2. Latex production (2002-2007)

3.3.2.1. Non-adjusted latex productions

Normally in industrial plantations, all the trees are opened at the same girth of the trunk (50 cm at 1-m high). By contrast, in small scale trials, for comparing the genotypes in similar conditions of the environment, early tapping is carried out on all the trees with a girth higher than 25 cm. Thus the productions of the genotypes are observed at varied mean girths. For comparing the productions at similar girth level, the production traits were adjusted by covariance analysis. But it was also interesting to apply QTL detection to the natural production data as well as to adjusted data. Therefore, QTL detection was carried out first for the non-adjusted BLUP data (Lp), and then for the girth-adjusted data (Lpa).

Table 48 presents the characteristics of the 14 QTLs detected for non-adjusted production traits. The most probable positions were g2-57, g3-60, g4-13, g5-24, g7-105, g8-24, g9-4, g11-104, g12-2, g13-19, g13-122, g16-6, g17-28, and g18-100.

Table 48 : Characteristics of the QTLs detected for latex production traits (MQM method). Value index 100 for genotypic class « ac ». Ranking by increasing linkage group number (chromosome number) and position (in cM).

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
Lp75	2	57	6.3	8	100	98	100	97
Lp74	2	57	4.8	4	100	98	101	98
Lp91	3	46	7.5	8	100	100	98	99
Lp74	3	47	6.2	5	100	101	97	99
Lp72	3	48	8.1	9	100	102	97	98
Lp83	3	49	7.2	9	100	101	98	98
Lp71	3	51	11.7	9	100	101	96	99
Lp73	3	51	9.7	10	100	101	97	99
Lp92	3	52	12.7	22	100	101	98	99
Lp81	3	52	7.3	6	100	101	97	100
Lp82	3	52	4.9	9	100	101	97	99
Lp96	3	59	9.6	15	100	100	97	99
Lp95	3	59	9.4	16	100	100	97	99
Lp94	3	59	8.2	18	100	101	98	100
Lp93	3	60	14.2	24	100	101	98	100
Lp91	4	13	6.9	7	100	102	100	101
Lp75	4	14	6.8	7	100	101	98	101
Lp71	4	18	5.2	5	100	101	99	103
Lp74	5	23	4.9	3	100	102	102	103

Lp81	5	24	5.5	4	100	102	104	101
Lp95	7	105	7.1	11	100	102	103	103
Lp96	7	105	8.8	13	100	102	102	102
Lp73	8	21	5.4	5	100	98	97	97
Lp71	8	24	9.5	6	100	98	96	96
Lp92	9	1	7.8	11	100	98	98	98
Lp93	9	1	5.4	8	100	99	99	99
Lp75	9	2	5.9	7	100	99	99	97
Lp71	9	9	6.9	3	100	100	99	96
Lp73	9	9	6.1	5	100	99	98	97
Lp74	9	10	8.8	8	100	99	98	96
Lp72	11	102	5.7	7	100	100	103	101
Lp74	11	103	4.8	4	100	99	101	99
Lp71	11	104	6.9	4	100	99	103	101
Lp73	11	106	5.5	5	100	99	102	100
Lp91	11	108	6.4	8	100	100	102	101
Lp81	11	109	5.2	4	100	100	103	101
Lp71	12	2	5.6	8	100	102	103	99
Lp75	13	18	4.9	5	100	102	102	102
Lp74	13	19	6.5	5	100	103	103	102
Lp72	13	113	6.0	9	100	98	101	103
Lp73	13	122	6.0	5	100	100	102	103
Lp74	13	123	4.6	3	100	100	101	103
Lp92	16	2	8.8	13	100	98	101	101
Lp71	16	5	37.1	35	100	96	99	103
Lp96	16	5	9.9	15	100	99	102	101
Lp81	16	6	45.5	56	100	96	109	101
Lp74	16	6	35.4	37	100	96	105	102
Lp73	16	6	30.9	39	100	97	104	102
Lp91	16	6	30.5	42	100	98	105	101
Lp83	16	6	23.9	37	100	97	104	101
Lp72	16	6	22.6	32	100	96	105	102
Lp75	16	6	22.3	29	100	97	104	102
Lp82	16	7	16.9	32	100	97	103	101
Lp95	16	7	10.5	18	100	99	102	101
Lp93	17	28	4.7	12	100	100	101	101
Lp93	18	93	4.9	7	100	100	99	99
Lp83	18	94	5.1	6	100	100	98	98
Lp71	18	100	6.2	4	100	102	100	99

The QTL on the chromosome g3 (from position g3-46 to g3-60) was detected for all the traits excepted Lp75. Its position and its effect (the class « bc » was always the lowest) indicate that it was the same QTL « g3-60 » that was detected for growth. This complies with the observed positive correlation between the production traits and the girth G7 (or G59).

One very important QTL was detected on g16 from position g16-2 to g16-7, with the most probable position on g16-6, for all the production traits excepted Lp93 and

Lp94. Its effect was maximum for Lp81 with LOD score = 45.5, and %exp = 56 %. For Lp92, Lp95 and Lp96, its effect was lower than for the other traits.

Figure 28 shows the curves of LOD score for the detection of the QTL g16-6 with its maximum effect in the case of the trait Lp81, by Interval Mapping method (IM) and by MQM method after putting the marker a131 (position g16-5) as cofactor in the analysis. The LOD support interval observed in the IM method, at 1 LOD unit below the peak, was from 4.8 to 7.8 cM. Therefore the « confidence interval » of the position of this QTL would be of only 3 cM. The MQM method showed that there was probably only one QTL for this trait on this chromosome.

Figure 28 : QTL g16-6. LOD scores observed from the analysis of the girth trait Lp81 by Interval Mapping (left) and MQM (right) methods. In the MQM method, the marker a131, positionned at locus g16-5 was here used as sole cofactor for this figure. Significance threshold LOD = 4.5 (horizontal dashed line).

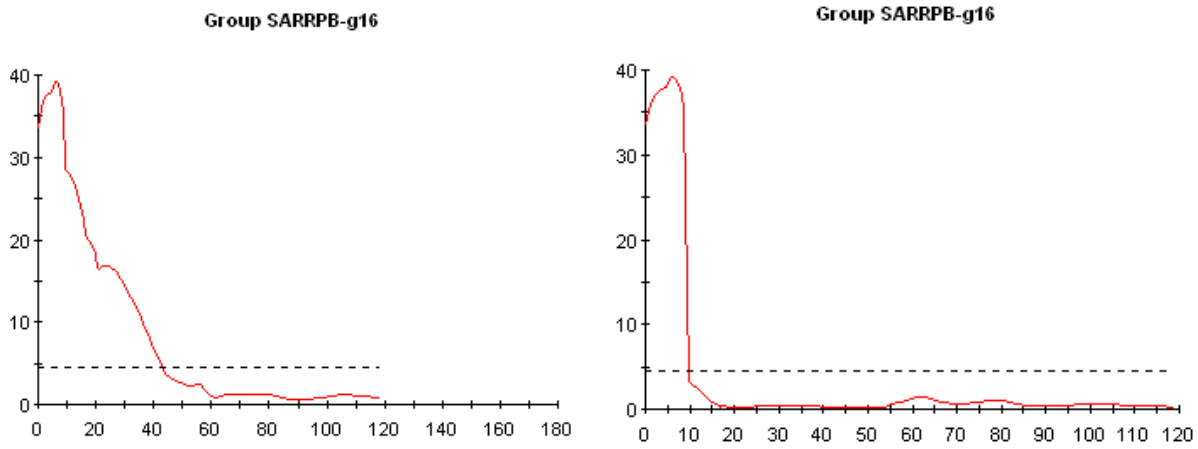
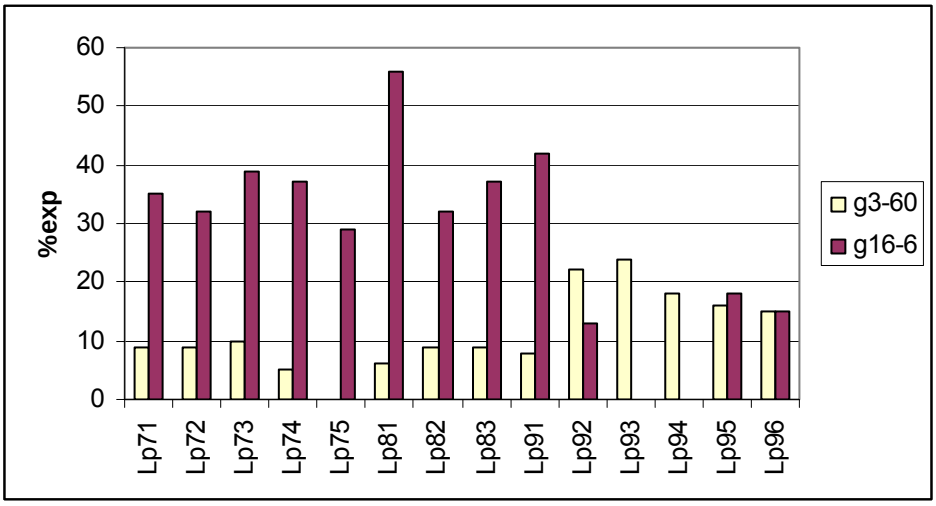


Figure 29 shows the percentages of explanation of the 14 production traits by the two QTLs g3-60 and g16-6. During the intensification of production, from Lp92 to Lp96, as compared to the preceding period, the %exp was higher for g3-60 and lower for g16-6. Moreover, the QTL g16-6 was not detected for the two production traits Lp93 and Lp94 after two stimulations. This figure shows that the QTL g3-60, detected from the production traits, had its highest effects on the traits observed during tapping intensification, from Lp92 to Lp96, with its maximum for LP93 after the first stimulation.

Figure 29 : Percentages of explanation (%exp) of the 14 non-adjusted production traits by the two QTLs g3-60 and g16-6.



For the QTL g16-6, the comparison of the four genotypic classes showed that, in all cases, the class « ad » was the lowest and the class « bc » was the highest, but with very small differences from Lp92 to Lp96.

The QTL on g7 (g7-105) was detected for Lp95 and Lp96, only at the end of the period of intensive exploitation from Lp92 to Lp96. The QTL on g17 (g17-28) was detected only for Lp93.

3.3.2.2. Girth-adjusted latex productions

Table 49 shows the characteristics of the QTLs detected for the production traits adjusted to the girth G7. Logically the QTL g3-60 was not detected, excepted for Lp95a, thus indicating an effect of the growth increment from May 2007 (date of G7 measurement) to September 2009 when Lp95 was measured. The QTLs detected for the non-adjusted traits were found again, excepted g11-104, g12-2, g13-19, and g17-28. Only one new QTL, g2-87, was detected for the adjusted productions.

Table 49 : Characteristics of the QTLs detected for girth-adjusted latex production traits (girth G7 put as covariable in BLUP estimations). Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
Lp94a	2	45	4.7	9	100	99	100	98
Lp73a	2	46	4.5	4	100	98	99	98
Lp71a	2	59	5.2	6	100	97	99	97
Lp92a	2	60	5.7	12	100	99	99	98
Lp74a	2	60	5.2	6	100	98	100	97
Lp91a	2	60	4.7	7	100	98	100	98
Lp82a	2	61	4.8	9	100	97	99	98
Lp75a	2	87	5.0	5	100	100	101	99
Lp95a	3	59	4.5	7	100	100	98	100
Lp91a	4	13	4.8	6	100	102	100	101
Lp75a	4	14	5.0	5	100	101	99	101
Lp72a	5	23	5.8	7	100	104	101	102
Lp73a	5	23	5.0	5	100	102	100	102
Lp81a	5	24	4.7	4	100	102	103	101
Lp95a	7	105	4.5	8	100	101	102	102
Lp96a	7	105	7.2	13	100	102	102	102
Lp73a	8	15	5.5	5	100	98	97	97
Lp71a	8	22	7.4	7	100	98	96	96
Lp92a	9	0	7.1	12	100	99	99	98
Lp94a	9	1	5.8	11	100	99	99	98
Lp93a	9	1	4.7	11	100	99	99	99
Lp75a	9	4	6.5	7	100	99	99	97
Lp73a	9	4	9.0	8	100	99	98	96
Lp72a	9	4	5.3	8	100	99	98	96
Lp74a	9	10	7.2	7	100	100	98	97
Lp71a	9	10	6.5	7	100	100	99	96
Lp73a	13	123	5.6	6	100	100	101	102
Lp74a	13	123	5.0	5	100	100	101	103
Lp92a	16	3	10.6	18	100	99	101	101
Lp81a	16	6	49.2	66	100	95	110	101
Lp74a	16	6	38.2	50	100	96	106	102
Lp71a	16	6	35.6	47	100	96	108	103
Lp91a	16	6	28.7	51	100	98	105	100
Lp72a	16	6	25.7	40	100	96	106	102
Lp96a	16	6	11.7	23	100	98	102	101
Lp94a	16	6	6.8	13	100	99	102	100
Lp73a	16	7	39.2	46	100	97	106	101
Lp75a	16	7	27.9	39	100	97	104	101
Lp83a	16	7	26.4	45	100	96	104	101
Lp82a	16	7	20.3	36	100	97	104	100
Lp95a	16	7	11.6	23	100	99	103	101
Lp83a	18	94	5.3	7	100	100	98	98

The QTL g16-6 explained a higher percentage of phenotypic variance for the adjusted production traits than for the non-adjusted traits (figure 30).

Figure 30 : QTL g16-6. Evolution of the percentage of explanation of the phenotypic variance of production traits, non-adjusted or adjusted on the girth G7. The QTL was not detected for Lp93 and LP94 (non-adjusted), nor for Lp93a (adjusted).

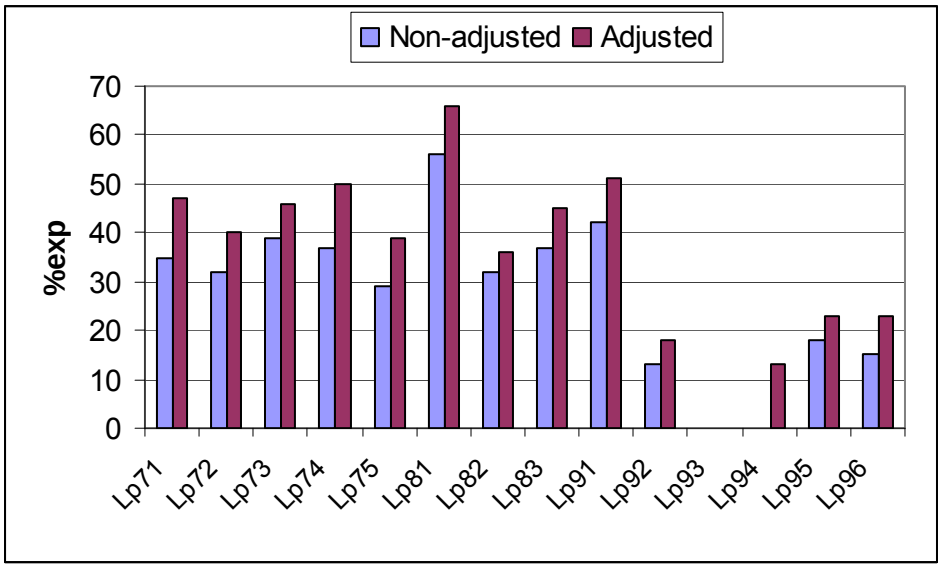


Table 50 summarizes the results of the percentages of explanation of the QTLs for the girth-adjusted production traits. The two QTLs g2-60 and g9-4 were the most frequently detected after g16-6. The lowest cumulated %exp were found for the traits observed under intensification (from Lp92a to Lp96a). The impact of the first stimulation in 2009 on Lp93a appeared to be very strong.

Table 50 : Percentages of explanation of the phenotypic variance of the genotypes by the 11 QTLs detected on the girth-adjusted production traits (adjustment on G7).

QTL	2-60	2-87	3-60	4-13	5-21	7-105	8-21	9-4	13-123	16-6	18-94	Total
Lp71a	6						7	7		47		67
Lp72a					7			8		40		55
Lp73a	4				5		5	8	6	46		74
Lp74a	6							7	5	50		68
Lp75a		5		5				7		39		56
Lp81a					4					66		70
Lp82a	9									36		45
Lp83a										45	7	52
Lp91a	7			6						51		64
Lp92a	12							12		18		42
Lp93a								11				11
Lp94a	9							11		13		33
Lp95a			7			8				23		38
Lp96a						13				23		36

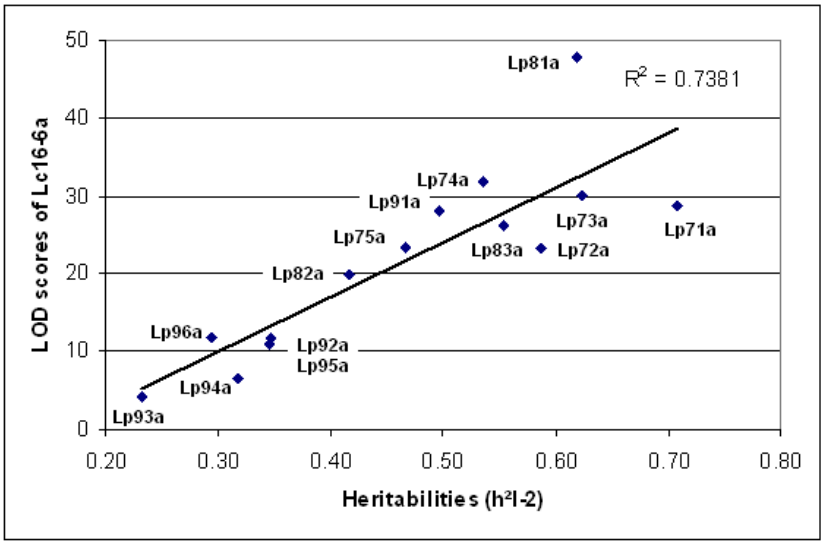
Table 51 shows the range of variation of the cumulated rubber production between the genotypic classes and between the extreme genotypes. These large differences indicate a high potential for molecular selection on this QTL.

Table 51 : Values of the cumulated rubber production (cg) from P71a to P96a (girth-adjusted traits) for the genotypic classes of the QTL g16-6, for RRIM600 and the extreme genotypes n° 169 and n° 32. Allelic classes of the nearest marker (a131).

QTL g16-6	Nb	Marker a131	Cp7196a	Index	Significance
bc	42	ee	330364	112	A
bd	51	eg	264806	90	B
ac	51	ef	248748	84	B
ad	52	fg	210864	72	C
N° 169 (Max)	1	ee	440667	150	-
RRIM600	1	ef	294735	100	-
n° 32 (Min)	1	fg	117984	40	-

Concerning the QTL g16-6, a clear positive correlation was shown between heritabilities and peak LOD scores (figure 31).

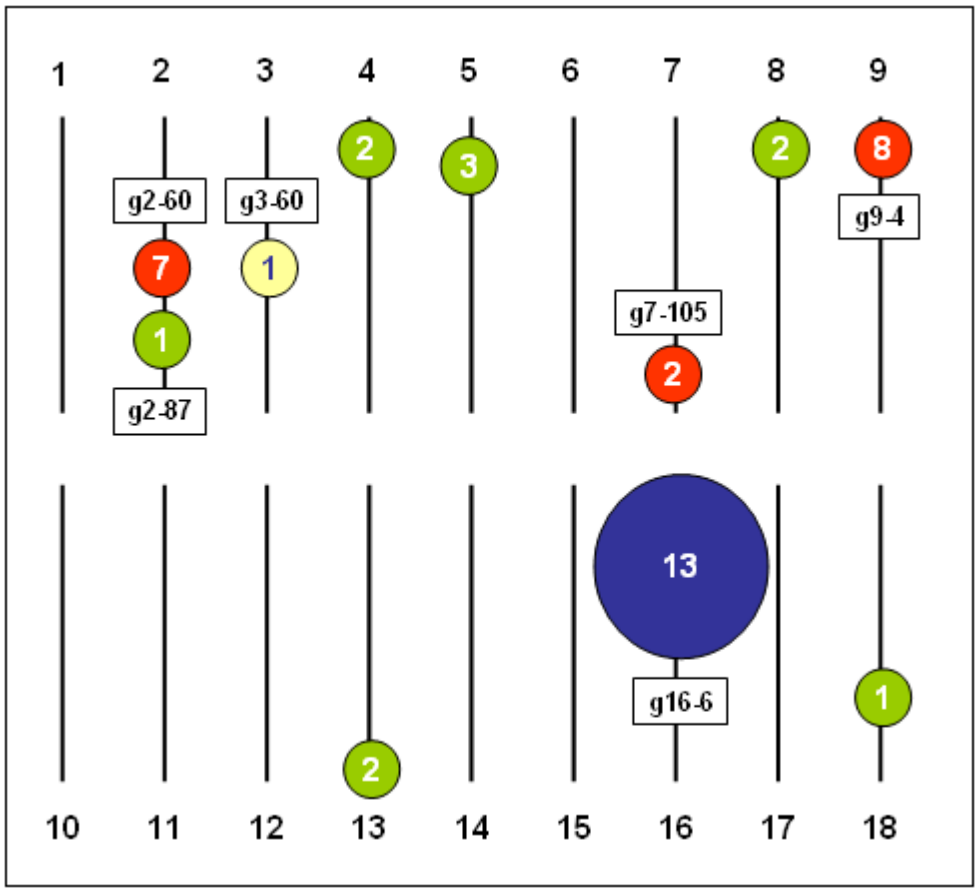
Figure 31 : Correlation between the heritabilities of the 14 girth-adjusted yield traits from Lp71a to Lp96a and the peak LOD scores estimated for the QTL Lc16-6 associated to these traits.



3.3.2.3. Summary of QTLs detected for production

Figure 32 recalls the positions of the 11 QTLs detected for the girth-adjusted production traits. Apart from the major QTL g16-6 which was detected for 13 among the 14 production traits, the two QTLs g9-4 and g2-60 were detected repeatedly for 8 and 7 traits respectively. The particular interest of the QTL g7-105 lies in the fact that it was detected for the two last traits P95 and P96, at the end of the intensification phase.

Figure 32 : Locations of the 11 QTLs detected for girth-adjusted production traits. The large circle refers to the major QTL g16-6. Numbers in the circles indicate the number of detections of the QTL among the 14 production traits.



3.3.3. Plugging index

Table 52 presents the results of QTL mapping for plugging index traits (W1, W2, Drc, PI) issued from the three series measured in 2007 (2 series) and 2009 (1 series). W1 and W2 data were log-transformed (LW1, LW2). Among the seven QTLs detected for these plugging index traits, five of them were already found for production traits, including g3-60 and g16-6. Two new QTLs were detected on positions g1-45 and g9-53 only for the trait LW296, which may indicate a specific response to intensive tapping. The QTL g3-60 (peak positions from 49 to 59 cM) was detected for W1 in the three series (LW173 LW174, LW196) and for PI96. The QTLs g9-0 and g8-21 were detected for W1 in the two series measured in 2007 (LW173 and LW174). The QTL g4-13 was detected for W2 in the two series measured in 2007 (LW273 and LW274), and for PI74. The QTL g16-6 (peak

positions from 5 to 6 cM) was detected for W2 in the two series measured in 2007 (LW273 and LW274) and for the two corresponding Drc traits DrcPI73 and DrcPI74. It was detected also for W1 measured in 2009 (LW196). It was detected for the three PI traits (PI73, PI74, and PI96), but its effect was lower for PI96.

Table 52 : QTLs detected for plugging index traits (LW1, LW2, Drc, PI) in ascending order of the groups and positions. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
LW296	1	45	4.6	10	100	100	100	100
LW174	3	49	4.9	9	100	100	97	99
LW173	3	59	11.7	20	100	101	96	99
LW196	3	59	7.0	13	100	100	97	98
PI96	3	60	5.3	9	100	97	91	93
LW274	4	13	7.8	8	100	104	98	102
LW273	4	13	5.3	6	100	103	98	101
PI74	4	15	5.5	6	100	86	110	88
LW173	8	21	5.2	9	100	98	97	97
LW174	8	21	5.0	9	100	99	98	97
LW173	9	0	4.6	8	100	99	98	97
LW174	9	0	4.6	8	100	98	98	97
LW296	9	53	6.3	21	100	100	100	100
LW196	16	5	8.8	16	100	101	98	101
PI96	16	5	14.8	26	100	107	91	103
PI74	16	6	37.9	58	100	153	64	100
PI73	16	6	37.9	61	100	144	68	101
LW274	16	6	30.8	49	100	92	107	101
LW273	16	6	28.2	47	100	93	107	102
DrcPI73	16	7	5.4	12	100	102	99	100
DrcPI74	16	7	14.8	31	100	104	95	98

3.3.4. Latex diagnostic

Table 53 provides the results of QTL detection for Drc traits, in the ascending order of the groups and positions. For all the Drc traits excepted Drc93, the QTL g16-6 was detected, with peak positions varying from 0 to 7 cM depending on the traits. The class « bc » was always the lowest for this QTL. Other QTLs were detected on groups 9, 12, and 17.

Table 53 : Characteristics of the QTLs detected for Drc traits (ascending order of the groups and positions). Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	Bd
Drc82	9	0	4.8	7	100	100	105	105
Drc72b	12	80	4.6	7	100	99	103	104
Drc96	16	0	4.8	9	100	101	98	102
Drc82	16	5	17.3	30	100	108	95	99
Drc74	16	6	23.9	44	100	106	92	99
Drc81	16	6	32.3	55	100	103	90	100
Drc72a	16	7	19.1	38	100	105	94	100
Drc72b	16	7	21.5	38	100	106	93	99
Drc92	16	7	12.3	26	100	104	94	99
Drc95	16	7	6.1	12	100	102	98	100
Drc96	17	63	5.1	11	100	100	103	100
Drc95	17	83	4.5	10	100	100	102	98

Table 54 provides the results from QTL detection for RSuc traits (root transformed data of sucrose traits). The QTL g16-6 was detected only for RSuc72a, RSuc72b, RSuc74, RSuc81, RSuc92, with the class « ad » being the highest and « bc » being the lowest. We can think that it was not possible to detect QTLs for traits from Rsuc93 to Rsuc96 due to the very small variance of these traits. A second distinct QTL was detected on group 16 for RSuc72b, at the peak position of 46 cM, with classes « bd » and « ac » being the highest.

Table 54 : Characteristics of the significant QTLs detected for RSuc traits (ascending order of the groups and positions). Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
RSuc72a	16	4	6.8	15	100	103	94	101
RSuc74	16	5	11.5	24	100	106	91	100
RSuc81	16	5	4.7	10	100	102	96	98
RSuc72b	16	6	4.8	10	100	104	98	100
RSuc92	16	7	8.0	18	100	104	98	100
RSuc72b	16	46	5.4	12	100	99	96	103

Table 55 provides the results from QTL detection for Pi traits (log transformed data for LPi81). The QTL g16-6 was detected for Pi72a, Pi72b, Pi74, LPi81, Pi82, Pi92, Pi93, and Pi96, but not for Pi95. The effect %exp was high (from 14 to 60 %)

excepted for Pi93 (%exp = 7 %). The genotypic class « bc » was the highest. For Pi93, four QTLs were detected at the most probable positions g2-126, g7-66, g12-48, and g18-94. The QTL at the end of the group 18 (from peak positions 90 to 100 cM) was detected for Pi82, Pi93, Pi95, and Pi96, which may indicate a genetic response to stimulation. Four other QTLs were detected at the positions g7-105 (Pi96), g8-21 (Pi92 and Pi95), g8-58 (Pi82), and g17-46 (Pi95).

Table 55 : Characteristics of the significant QTLs detected for Pi traits (ascending order of the groups and positions). Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
Pi93	2	126	6.6	10	100	98	101	111
Pi93	7	66	5.6	8	100	92	102	102
Pi96	7	105	8.8	15	100	105	115	112
Pi92	8	21	4.5	8	100	93	89	83
Pi95	8	21	5.5	11	100	91	95	91
Pi82	8	58	5.9	9	100	96	85	85
Pi93	12	48	4.8	10	100	90	102	97
Pi96	16	4	8.1	14	100	101	114	108
Pi82	16	5	19.8	32	100	87	124	110
Pi92	16	5	11.3	21	100	91	119	104
Pi93	16	5	5.5	7	100	99	110	105
LPi81	16	6	37.5	60	100	96	128	103
Pi72a	16	6	22.9	44	100	87	134	111
Pi72b	16	6	23.7	44	100	84	125	106
Pi74	16	6	10.8	23	100	90	117	102
Pi95	17	46	4.7	11	100	99	102	108
Pi82	18	90	5.3	8	100	102	89	87
Pi93	18	94	9.7	14	100	96	90	84
Pi95	18	98	7.9	15	100	98	96	89
Pi96	18	100	4.5	8	100	99	96	90

Table 56 provides the results from QTL detection for Rsh traits, in the ascending order of the groups (chromosomes) and positions.

Table 56 : Characteristics of the significant QTLs detected for Rsh traits (ascending order of the groups and positions). Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
Rsh92	1	5	5.4	12	100	113	108	104
Rsh93	1	66	7.3	16	100	103	107	120
Rsh96	1	68	5.2	12	100	100	104	111
Rsh81	1	77	4.5	11	100	108	112	115
Rsh74	1	80	4.7	10	100	108	108	108
Rsh82	1	80	10.7	22	100	107	107	119
Rsh92	1	80	6.3	14	100	104	108	113
Rsh95	1	85	6	15	100	108	113	117
Rsh72b	3	60	4.6	9	100	97	106	97
Rsh93	11	35	5.3	11	100	87	97	87
Rsh72b	12	90	4.5	10	100	90	94	90

The QTL g1-80 (peak positions varying from 66 to 85 cM) was detected repefor Rsh74, Rsh81, Rsh82, Rsh92, Rsh93, Rsh95, and Rsh96, with the class « bd » being the highest. The QTL g3-60 was detected for Rsh72b, with the class « bc » being the highest. Other QTLs were detected at peak positions g1-5 (Rsh92), g11-35 (Rsh93), and g12-90 (Rsh72b).

3.3.5. Defoliation earliness

Table 57 shows the characteristics of the QTLs detected for the traits related with defoliation earliness from 2005 to 2009. A total of 8 different QTLs were detected for these defoliation traits.

No QTL was detected for any of the two traits relative to the year 2007 (Def55a and Def55b). Five QTLs were detected only for one trait. The QTLs g3-108 and g5-21 were detected for Def31 (2005). The QTL g10-120 was detected for Def43 (2006). The QTL g3-34 was detected for Def66c (2008). The QTL g4-77 was detected for Def79c (2009). The QTL g12-30 (positions 28 and 32) was detected in for Def31 (2005) and Def79d (2009). The QTL g17-30 was detected for Def66a and Def66e (2008) and Def79c (2009). The QTL g10-68 (positions observed from 66 to 71 cM) was detected for six traits in 2006, 2008, and 2009.

Table 57 : Characteristics of the QTLs detected for annual leaf-defoliation period. (ascending order of the groups and positions). Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Year	Group	Position	LOD	%exp	ac	ad	bc	bd
Def66c	2008	3	34	4.6	12	100	102	94	97
Def31	2005	3	108	5.1	9	100	122	105	91
Def79c	2009	4	77	5.2	14	100	100	112	109
Def31	2005	5	21	7	13	100	92	121	103
Def79a	2009	10	66	5.4	15	100	101	98	108
Def79b	2009	10	68	5.7	17	100	114	104	118
Def66e	2008	10	69	4.7	14	100	107	101	108
Def79c	2009	10	69	5.2	16	100	115	109	116
Def43	2006	10	70	4.5	11	100	125	115	133
Def66b	2008	10	71	4.5	11	100	104	102	107
Def43	2006	10	120	5	10	100	108	128	129
Def31	2005	12	28	6.2	11	100	111	110	128
Def79d	2009	12	32	6.8	21	100	99	101	91
Def79c	2009	17	29	4.6	11	100	95	106	106
Def66a	2008	17	30	5.4	12	100	96	109	109
Def66e	2008	17	30	5.3	12	100	95	103	104

3.3.6. Leaves dimensions

Two QTLs were detected for leaves dimensions : the QTL g3-60 normally associated to growth fastness, and the QTL g7-20 which was here associated to the width of the leaflets (table 58).

Table 58 : Characteristics of the QTLs detected for leaves dimensions. Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	Ac	ad	bc	bd
FW11	3	62	6.6	16	100	101	90	98
FW12	3	65	8.9	21	100	101	89	98
FW13	3	64	7.8	19	100	100	89	98
FW1 (mean)	3	64	8.1	19	100	100	88	98
L12	3	61	6	14	100	100	95	98
W12	7	20	5.2	14	100	96	98	97
W13	7	20	5.2	14	100	96	98	96
FW21	3	69	4.6	13	100	99	95	99
FW22	3	67	5.8	16	100	99	92	98
FW2	3	69	5.1	14	100	99	94	99
L1 (mean)	3	60	5	11	100	100	95	98
W1 (mean)	7	20	4.9	13	100	96	98	96

3.3.7. Bark thickness

Among the QTLs detected for non-adjusted bark thickness traits, presented in table 59, the QTL g3-60, normally associated with girth, was found for B1 and Bm. Another QTL, g3-18, was found on the group g3 for B5p and B6p. The QTL g16-6, normally associated with latex production, was found for B2p, B3, and B4, with peak positions varying from 1 to 7 cM.

The QTL g5-21 was significant for B1, B3, and Bm, with peak positions varying from 17 to 21 cM. The QTL g16-60 was significant only for Bm but near the threshold for B2p and B4.

Table 59 : Characteristics of QTLs detected for non-adjusted bark thickness traits. Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
B1	5	21	5.7	13	100	82	112	110
B1	3	59	4.6	9	100	101	76	88
B2p	16	1	5.4	13	100	142	96	106
B2p	16	62	4.3	8	100	104	67	88
B3	16	2	6.7	17	100	145	86	95
B3	5	20	4.2	8	100	79	121	100
B4	16	7	6.6	15	100	139	79	98
B4	16	62	4.4	9	100	112	68	88
B5p	3	18	4.7	11	100	79	61	65
B5p	5	21	3.5	8	100	74	96	100
B6p	3	18	5.1	12	100	79	61	65
B6p	11	92	3.6	11	100	100	95	69
Bm	5	17	6.6	12	100	96	101	99
Bm	3	60	6.1	11	100	100	96	98
Bm	16	42	5.9	12	100	102	97	100

QTLs detected for girth-adjusted bark thickness traits are presented in table 60. Logically, the QTL g3-60 was no more found ; moreover, the QTL g3-18 was below significance threshold for B5pa and B6pa. The QTL g16-6 was found again for B2pa, B3a, B4a, and also Bma. The QTLs g5-21 was found significant only for B1a. The QTL g16-60 was found significant for B4a and Bma.

Table 60 : Characteristics of QTLs detected for girth-adjusted bark thickness traits. Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
B1a	5	20	5.1	12	100	99	100	101
B1a	13	5	4.3	10	100	99	99	98
B2pa	16	60	4.3	9	100	101	98	99
B2pa	16	1	4.1	9	100	102	100	100
B3a	16	1	6.1	15	100	103	99	99
B3a	16	66	3.7	7	100	101	98	99
B4a	16	1	5.6	13	100	103	99	99
B4a	16	61	5.4	11	100	102	98	99
B4a	7	99	3.9	7	100	97	97	97
B5pa	16	61	4.1	10	100	101	97	100
B5pa	5	21	3.5	9	100	97	99	100
B5pa	6	102	3.3	18	100	100	105	100
B5pa	3	18	3.1	7	100	98	97	97
B6pa	16	62	4.0	9	100	101	97	100
B6pa	3	18	3.4	8	100	98	96	97
Bma	16	60	5.8	14	100	102	97	100
Bma	16	0	3.6	8	100	103	100	99
Bma	7	101	3.4	7	100	98	97	98
Bma	5	20	3.2	6	100	98	101	100

3.3.8. Die-back

As shown in table 61, only one QTL, g18-92, could be considered as significant.

Table 61 : QTL associated with the variation of the die-back index (June 2005). Detection by Kruskal-Wallis method for adapting to a non-normal distribution of the die-back index. Position in cM. Value index 100 for the genotypic class « ac ».

QTL	Marker	Segregation	Kobs	Df	p-value	ac	ad	bc	bd
Lc18-92	g18MT65	<ab x cd>	14.334	3	<0.005	100	73	82	125

3.3.9. Molecular mass distributions

Table 62 shows the results of QTL detection for the traits characterizing the molar mass distributions of rubber.

Table 62 : Characteristics of the QTLs detected for molar mass distribution traits. Positions in cM. Value index 100 for genotypic class « ac ».

Trait	Group	Position	LOD	%exp	ac	ad	bc	bd
M_n	3	59	4.5	9	100	98	89	97
M_{z+1}	3	65	5.4	12	100	101	100	100
M_z	3	65	4.8	11	100	101	99	100
R21	3	69	4.6	9	100	95	111	106
R21	8	81	6.3	10	100	91	99	109
R21	10	85	5.3	10	100	89	99	105
R21	14	50	6.9	13	100	106	113	119
R21	15	53	7.1	13	100	115	114	117
M_n	16	1	5.4	12	100	108	96	100
I_p	16	5	11.3	23	100	92	104	100
Gel	16	7	7.6	17	100	104	97	99

The QTL g3-60 (growth in girth), was detected for the molar mass traits M_{z+1}, M_z, and M_n, and the ratio of short rubber chains R21. The class « bc » of the QTL was the lowest for M_n, in accordance with the positive correlation between M_n and G59.

The QTL g16-6 (production) was detected for Gel, I_p, and M_n. The class « bc » of the QTL was the lowest for Mn, in accordance with the negative correlation between Mn and Lpcum0708a.

The four QTLs g8-81, g10-85, g14-50, and g15-53 were detected for R21, the ratio of short chains. Therefore, there were 5 QTLs (including also g3-60) contributing to the genetic determinism of R21, with a cumulated percentage of explanation of the phenotypic variance of 55 %.

3.4. Synthesis about QTL detection

A total number of 226 cases of QTL detection were observed. These results were gathered and ordered in increasing number of the chromosomes and of the positions on each chromosome (table 63). Thus 48 chromosomic segments were identified as QTLs. Although the delimitation of these QTLs was sometimes questionable, we considered that each segment was equivalent to one QTL, and it was named after one position. The choice of the position was based on the frequency of the position, on the LOD scores and the percentages of explanation of the phenotypic variance. For differentiating two types of traits, we sometimes accepted to consider two QTLs at two close positions, for example g5-16 for Hi1723 and g5-21 for growth during tapping, or g12-20 for Gai1218 and g12-32 for Gai5359 and biomass, or g16-42 for the bark thickness trait Bm, and g16-46 for the sucrose content trait RSuc72b. The largest ranges of positions for one QTL were of 20 cM (g3-60 and g17-63). In fact, concerning the QTL g3-60, if we don't take into account the position g3-49, the range of variation of g3-60 goes from g3-59 to g3-69 ; among these 47 cases, 37 cases were in the interval from g3-59 to g3-62.

Table 63 : Localisation of the 226 cases of QTL detection. Increasing order of chromosome numbers and of positions (in centimorgans) on each chromosome.

Type of trait	Trait	QTL	Group	Position	LOD	%exp
Latex diagnostic	Rsh92	g1-5	1	5	5.4	12
Plugging index	LW296	g1-45	1	45	4.6	10
Latex diagnostic	Rsh93	g1-80	1	66	7.3	16
Latex diagnostic	Rsh96		1	68	5.2	12
Latex diagnostic	Rsh81		1	77	4.5	11
Latex diagnostic	Rsh82		1	80	10.7	22
Latex diagnostic	Rsh92		1	80	6.3	14
Latex diagnostic	Rsh74		1	80	4.7	10
Latex diagnostic	Rsh95		1	85	6.0	15
Production	Lp94a	g2-60	2	45	4.7	9
Production	Lp73a		2	46	4.5	4
Production	Lp71a		2	59	5.2	6
Production	Lp92a		2	60	5.7	12
Production	Lp74a		2	60	5.2	6
Production	Lp91a		2	60	4.7	7
Production	Lp82a		2	61	4.8	9
Production	Lp75a	g2-87	2	87	5.0	5
Latex diagnostic	Pi93	g2-126	2	126	6.6	10

Bark thickness	B6p	g3-18	3	18	5.1	12
Bark thickness	B5p		3	18	4.7	11
Defoliation earliness	Def66c	g3-34	3	34	4.6	12
Plugging index	LW174	g3-60	3	49	4.9	9
Plugging index	LW173		3	59	11.7	20
Plugging index	LW196		3	59	7.0	13
Growth before tapping	H53		3	59	6.0	14
Growth before tapping	Gi4347		3	59	5.3	12
Bark thickness	B1		3	59	4.6	9
Production	Lp95a		3	59	4.5	7
Molar mass distributions	Mn		3	59	4.5	9
Growth during tapping	G85		3	60	21.7	30
Growth during tapping	G82		3	60	21.6	30
Growth during tapping	G89		3	60	21.2	30
Growth during tapping	G76		3	60	19.5	27
Growth during tapping	G71		3	60	17.4	28
Growth during tapping	G64		3	60	16.0	25
Growth before tapping	G59		3	60	15.8	31
Growth before tapping	G53		3	60	13.4	27
Growth before tapping	G47		3	60	12.4	26
Growth before tapping	Gi5359		3	60	9.7	18
Growth before tapping	Ga23		3	60	9.5	20
Growth before tapping	Gai1218		3	60	9.4	18
Growth before tapping	Ga18		3	60	9.1	18
Growth before tapping	G43		3	60	9.1	19
Growth before tapping	Gi4753		3	60	8.4	18
Growth before tapping	G31		3	60	8.0	17
Growth before tapping	G23		3	60	7.3	16
Growth during tapping	Gi8285		3	60	7.3	16
Growth before tapping	G18		3	60	6.9	15
Growth before tapping	G36		3	60	6.5	14
Bark thickness	Bm		3	60	6.1	11
Growth during tapping	Gi5964		3	60	5.8	9
Plugging index	PI96		3	60	5.3	9
Leaves dimensions	L1 (mean)		3	60	5.0	11
Latex diagnostic	Rsh72b		3	60	4.6	9
Die-back	DB-index		3	60	p<0.005	-
Growth during tapping	G79		3	61	21.6	31
Growth during tapping	Gi8289		3	61	7.9	17
Leaves dimensions	L12		3	61	6.0	14
Leaves dimensions	FW11		3	62	6.6	16
Leaves dimensions	FW1 (mean)		3	64	8.1	19
Leaves dimensions	FW13		3	64	7.8	19
Leaves dimensions	FW12		3	65	8.9	21
Molar mass distributions	Mz+1		3	65	5.4	12
Molar mass distributions	Mz		3	65	4.8	11
Leaves dimensions	FW22		3	67	5.8	16
Leaves dimensions	FW2		3	69	5.1	14
Leaves dimensions	FW21		3	69	4.6	13
Molar mass distributions	R21		3	69	4.6	9
Defoliation earliness	Def31	g3-108	3	108	5.1	9
Plugging index	LW274	g4-13	4	13	7.8	8

Plugging index	LW273		4	13	5.3	6
Production	Lp91a		4	13	4.8	6
Production	Lp75a		4	14	5.0	5
Plugging index	PI74		4	15	5.5	6
Defoliation earliness	Def79c	g4-77	4	77	5.2	14
Growth before tapping	Hi1723	g5-16	5	16	4.9	11
Bark thickness	Bm	g5-21	5	17	6.6	12
Bark thickness	B1a		5	20	5.1	12
Defoliation earliness	Def31		5	21	7.0	13
Bark thickness	B1		5	21	5.7	13
Growth during tapping	G76		5	22	5.5	7
Growth during tapping	G79		5	22	4.7	6
Growth during tapping	G85		5	22	4.6	6
Growth during tapping	G89		5	22	4.6	6
Growth during tapping	G64		5	22	4.5	6
Production	Lp72a		5	23	5.8	7
Production	Lp73a		5	23	5.0	5
Growth during tapping	G82		5	23	4.8	6
Production	Lp81a		5	24	4.7	4
Leaves dimensions	W12	g7-20	7	20	5.2	14
Leaves dimensions	W13		7	20	5.2	14
Leaves dimensions	W1 (mean)		7	20	4.9	13
Latex diagnostic	Pi93	g7-66	7	66	5.6	8
Latex diagnostic	Pi96	g7-105	7	105	8.8	15
Production	Lp96a		7	105	7.2	13
Growth before tapping	H47		7	105	5.2	11
Production	Lp95a		7	105	4.5	8
Production	Lp73a	g8-21	8	15	5.5	5
Latex diagnostic	Pi95		8	21	5.5	11
Plugging index	LW173		8	21	5.2	9
Plugging index	LW174		8	21	5.0	9
Latex diagnostic	Pi92		8	21	4.5	8
Production	Lp71a		8	22	7.4	7
Latex diagnostic	Pi82	g8-58	8	58	5.9	9
Die-back	DB-index	g8-73	8	73	p<0.005	-
Molar mass distributions	R21	g8-81	8	81	6.3	10
Growth before tapping	H30	g8-88	8	84	5.7	13
Production	Lp92a	g9-4	9	0	7.1	12
Latex diagnostic	Drc82		9	0	4.8	7
Plugging index	LW173		9	0	4.6	8
Plugging index	LW174		9	0	4.6	8
Production	Lp94a		9	1	5.8	11
Production	Lp93a		9	1	4.7	11
Production	Lp73a		9	4	9.0	8
Production	Lp75a		9	4	6.5	7
Production	Lp72a		9	4	5.3	8
Production	Lp74a		9	10	7.2	7
Production	Lp71a		9	10	6.5	7
Growth during tapping	H79	g9-26	9	26	6.0	17
Plugging index	LW296	g9-53	9	53	6.3	21

Defoliation earliness	Def79a	g10-68	10	66	5.4	15
Defoliation earliness	Def79b		10	68	5.7	17
Defoliation earliness	Def79c		10	69	5.2	16
Defoliation earliness	Def66e		10	69	4.7	14
Defoliation earliness	Def43		10	70	4.5	11
Defoliation earliness	Def66b		10	71	4.5	11
Molar mass distributions	R21	g10-85	10	85	5.3	10
Growth before tapping	Hi2330	g10-100	10	100	5.2	11
Defoliation earliness	Def43	g10-120	10	120	5.0	10
Latex diagnostic	Rsh93	g11-35	11	35	5.3	11
Biomass	Biom5359	g11-102	11	102	4.6	7
Growth before tapping	Gai1218	g12-32	12	20	5.6	12
Defoliation earliness	Def31		12	28	6.2	11
Growth before tapping	Gi5359		12	32	7.7	16
Biomass	Biom5359		12	33	6.5	11
Defoliation earliness	Def79d		12	32	6.8	21
Latex diagnostic	Pi93	g12-48	12	48	4.8	10
Latex diagnostic	Drc72b	g12-80	12	80	4.6	7
Latex diagnostic	Rsh72b	g12-90	12	90	4.5	10
Production	Lp73a	g13-123	13	123	5.6	6
Production	Lp74a		13	123	5.0	5
Molar mass distributions	R21	g14-50	14	50	6.9	13
Molar mass distributions	R21	g15-53	15	53	7.1	13
Latex diagnostic	Drc96	g16-6	16	0	4.8	9
Bark thickness	B3a		16	1	6.1	15
Bark thickness	B4a		16	1	5.6	13
Bark thickness	B2p		16	1	5.4	13
Molar mass distributions	Mn		16	1	5.4	12
Bark thickness	B3		16	2	6.7	17
Production	Lp92a		16	3	10.6	18
Growth during tapping	Gi7176		16	3	9.1	20
Latex diagnostic	Pi96		16	4	8.1	14
Latex diagnostic	RSuc72a		16	4	6.8	15
Latex diagnostic	Pi82		16	5	19.8	32
Latex diagnostic	Drc82		16	5	17.3	30
Plugging index	PI96		16	5	14.8	26
Latex diagnostic	RSuc74		16	5	11.5	24
Latex diagnostic	Pi92		16	5	11.3	21
Molar mass distributions	Ip		16	5	11.3	23
Plugging index	LW196		16	5	8.8	16
Latex diagnostic	Pi93		16	5	5.5	7
Latex diagnostic	RSuc81		16	5	4.7	10
Production	Lp81a		16	6	49.2	66
Production	Lp74a		16	6	38.2	50
Plugging index	PI74		16	6	37.9	58
Plugging index	PI73		16	6	37.9	61
Latex diagnostic	Pi81		16	6	37.5	60

Production	Lp71a		16	6	35.6	47
Latex diagnostic	Drc81		16	6	32.3	55
Plugging index	LW274		16	6	30.8	49
Production	Lp91a		16	6	28.7	51
Plugging index	LW273		16	6	28.2	47
Production	Lp72a		16	6	25.7	40
Latex diagnostic	Drc74		16	6	23.9	44
Latex diagnostic	Pi72b		16	6	23.7	44
Latex diagnostic	Pi72a		16	6	22.9	44
Production	Lp96a		16	6	11.7	23
Latex diagnostic	Pi74		16	6	10.8	23
Production	Lp94a		16	6	6.8	13
Latex diagnostic	RSuc72b		16	6	4.8	10
Production	Lp73a		16	7	39.2	46
Production	Lp75a		16	7	27.9	39
Production	Lp83a		16	7	26.4	45
Latex diagnostic	Drc72b		16	7	21.5	38
Production	Lp82a		16	7	20.3	36
Latex diagnostic	Drc72a		16	7	19.1	38
Growth during tapping	Gi5964		16	7	17.0	31
Plugging index	DrcPI74		16	7	14.8	31
Latex diagnostic	Drc92		16	7	12.3	26
Production	Lp95a		16	7	11.6	23
Latex diagnostic	RSuc92		16	7	8.0	18
Molar mass distributions	Gel		16	7	7.6	17
Bark thickness	B4		16	7	6.6	15
Latex diagnostic	Drc95		16	7	6.1	12
Plugging index	DrcPI73		16	7	5.4	12
Growth before tapping	Gai1218		16	7	4.6	7
Growth during tapping	G76		16	8	12.6	16
Growth during tapping	G79		16	8	11.6	15
Growth during tapping	G82		16	8	11.4	15
Growth during tapping	G85		16	8	10.6	13
Growth during tapping	G64		16	9	12.0	19
Growth during tapping	G71		16	9	10.0	15
Growth during tapping	G89		16	9	9.8	12
Bark thickness	Bm	g16-42	16	42	5.9	12
Latex diagnostic	RSuc72b	g16-46	16	46	5.4	12
Bark thickness	Bma	g16-60	16	60	5.8	14
Bark thickness	B4a		16	61	5.4	11
Defoliation earliness	Def79c	g17-30	17	29	4.6	11
Defoliation earliness	Def66a		17	30	5.4	12
Defoliation earliness	Def66e		17	30	5.3	12
Latex diagnostic	Pi95	g17-46	17	46	4.7	11
Latex diagnostic	Drc96	g17-63	17	63	5.1	11
Latex diagnostic	Drc95		17	83	4.5	10
Growth before tapping	Hi3643	g17-84	17	84	4.7	12
Growth during tapping	G89	g18-59	18	57	5.6	6
Growth during tapping	G79		18	57	5.0	6
Growth during tapping	G85		18	59	5.1	6
Growth during tapping	G82		18	59	5.0	6
Growth during tapping	G76		18	59	4.5	5

Growth during tapping	Gi8289		18	60	4.6	9
Latex diagnostic	Pi82	g18-94	18	90	5.3	8
Die-back	DB-index		18	92	p<0.005	-
Latex diagnostic	Pi93		18	94	9.7	14
Production	Lp83a		18	94	5.3	7
Latex diagnostic	Pi95		18	98	7.9	15
Latex diagnostic	Pi96		18	100	4.5	8

Table 64 shows the ranking of the 226 cases of QTL detection in decreasing order of the percentage of explanation of the phenotypic variances of the traits. The 21 cases with %exp varying from 66 to 32 % were related with the QTL g16-6. The QTL with the most important effect coming after was g3-60.

Table 64 : Ranking of the associations QTL-trait in the decreasing order of %exp.

Trait	Group	Position	LOD	%exp		Trait	Group	Position	LOD	%exp
Lp81a	16	6	49.2	66		Def79d	12	32	6.8	21
PI73	16	6	37.9	61		Pi92	16	5	11.3	21
Pi81	16	6	37.5	60		LW173	3	59	11.7	20
PI74	16	6	37.9	58		Ga23	3	60	9.5	20
Drc81	16	6	32.3	55		Gi7176	16	3	9.1	20
Lp91a	16	6	28.7	51		G43	3	60	9.1	19
Lp74a	16	6	38.2	50		FW1 (mean)	3	64	8.1	19
LW274	16	6	30.8	49		FW13	3	64	7.8	19
Lp71a	16	6	35.6	47		G64	16	9	12	19
LW273	16	6	28.2	47		Gi5359	3	60	9.7	18
Lp73a	16	7	39.2	46		Gai1218	3	60	9.4	18
Lp83a	16	7	26.4	45		Ga18	3	60	9.1	18
Drc74	16	6	23.9	44		Gi4753	3	60	8.4	18
Pi72b	16	6	23.7	44		Lp92a	16	3	10.6	18
Pi72a	16	6	22.9	44		RSuc92	16	7	8	18
Lp72a	16	6	25.7	40		G31	3	60	8	17
Lp75a	16	7	27.9	39		Gi8289	3	61	7.9	17
Drc72b	16	7	21.5	38		H79	9	26	6	17
Drc72a	16	7	19.1	38		Def79b	10	68	5.7	17
Lp82a	16	7	20.3	36		B3	16	2	6.7	17
Pi82	16	5	19.8	32		Gel	16	7	7.6	17
G59	3	60	15.8	31		Rsh93	1	66	7.3	16
G79	3	61	21.6	31		G23	3	60	7.3	16
Gi5964	16	7	17	31		Gi8285	3	60	7.3	16
DrcPI74	16	7	14.8	31		FW11	3	62	6.6	16
G85	3	60	21.7	30		FW22	3	67	5.8	16
G82	3	60	21.6	30		Def79c	10	69	5.2	16
G89	3	60	21.2	30		Gi5359	12	32	7.7	16
Drc82	16	5	17.3	30		LW196	16	5	8.8	16
G71	3	60	17.4	28		G76	16	8	12.6	16
G76	3	60	19.5	27		Rsh95	1	85	6	15
G53	3	60	13.4	27		G18	3	60	6.9	15
G47	3	60	12.4	26		Pi96	7	105	8.8	15
PI96	16	5	14.8	26		Def79a	10	66	5.4	15
Drc92	16	7	12.3	26		B3a	16	1	6.1	15
G64	3	60	16	25		RSuc72a	16	4	6.8	15
RSuc74	16	5	11.5	24		B4	16	7	6.6	15
Ip	16	5	11.3	23		G79	16	8	11.6	15
Lp96a	16	6	11.7	23		G82	16	8	11.4	15
Pi74	16	6	10.8	23		G71	16	9	10	15
Lp95a	16	7	11.6	23		Pi95	18	98	7.9	15
Rsh82	1	80	10.7	22		Rsh92	1	80	6.3	14
FW12	3	65	8.9	21		H53	3	59	6	14
LW296	9	53	6.3	21		G36	3	60	6.5	14

Table 64 : (Following) Ranking of the associations QTL-trait in the decreasing order of %exp.

Trait	Group	Position	LOD	%exp		Trait	Group	Position	LOD	%exp
L12	3	61	6	14		Bm	3	60	6.1	11
FW2	3	69	5.1	14		L1 (mean)	3	60	5	11
Def79c	4	77	5.2	14		Mz	3	65	4.8	11
W12	7	20	5.2	14		Hi1723	5	16	4.9	11
W13	7	20	5.2	14		H47	7	105	5.2	11
Def66e	10	69	4.7	14		Pi95	8	21	5.5	11
Pi96	16	4	8.1	14		Lp94a	9	1	5.8	11
Bma	16	60	5.8	14		Lp93a	9	1	4.7	11
Pi93	18	94	9.7	14		Def43	10	70	4.5	11
LW196	3	59	7	13		Def66b	10	71	4.5	11
FW21	3	69	4.6	13		Hi2330	10	100	5.2	11
Def31	5	21	7	13		Rsh93	11	35	5.3	11
B1	5	21	5.7	13		Def31	12	28	6.2	11
W1 (mean)	7	20	4.9	13		Biom5359	12	33	6.5	11
Lp96a	7	105	7.2	13		B4a	16	61	5.4	11
H30	8	84	5.7	13		Def79c	17	29	4.6	11
R21	14	50	6.9	13		Pi95	17	46	4.7	11
R21	15	53	7.1	13		Drc96	17	63	5.1	11
B4a	16	1	5.6	13		LW296	1	45	4.6	10
B2p	16	1	5.4	13		Rsh74	1	80	4.7	10
Lp94a	16	6	6.8	13		Pi93	2	126	6.6	10
G85	16	8	10.6	13		R21	8	81	6.3	10
Rsh92	1	5	5.4	12		R21	10	85	5.3	10
Rsh96	1	68	5.2	12		Def43	10	120	5	10
Lp92a	2	60	5.7	12		Pi93	12	48	4.8	10
B6p	3	18	5.1	12		Rsh72b	12	90	4.5	10
Def66c	3	34	4.6	12		RSuc81	16	5	4.7	10
Gi4347	3	59	5.3	12		RSuc72b	16	6	4.8	10
Mz+1	3	65	5.4	12		Drc95	17	83	4.5	10
Bm	5	17	6.6	12		Lp94a	2	45	4.7	9
B1a	5	20	5.1	12		Lp82a	2	61	4.8	9
Lp92a	9	0	7.1	12		LW174	3	49	4.9	9
Gai1218	12	20	5.6	12		B1	3	59	4.6	9
Mn	16	1	5.4	12		Mn	3	59	4.5	9
Drc95	16	7	6.1	12		Gi5964	3	60	5.8	9
DrcPI73	16	7	5.4	12		PI96	3	60	5.3	9
G89	16	9	9.8	12		Rsh72b	3	60	4.6	9
Bm	16	42	5.9	12		R21	3	69	4.6	9
RSuc72b	16	46	5.4	12		Def31	3	108	5.1	9
Def66a	17	30	5.4	12		LW173	8	21	5.2	9
Def66e	17	30	5.3	12		LW174	8	21	5	9
Hi3643	17	84	4.7	12		Pi82	8	58	5.9	9
Rsh81	1	77	4.5	11		Drc96	16	0	4.8	9
B5p	3	18	4.7	11		Gi8289	18	60	4.6	9

Table 64 : (Following and end) Ranking of the associations QTL-trait in the decreasing order of %exp.

Trait	Group	Position	LOD	%exp		Trait	Group	Position	LOD	%exp
LW274	4	13	7.8	8		Lp74a	2	60	5.2	6
Pi93	7	66	5.6	8		LW273	4	13	5.3	6
Lp95a	7	105	4.5	8		Lp91a	4	13	4.8	6
Pi92	8	21	4.5	8		PI74	4	15	5.5	6
LW173	9	0	4.6	8		G79	5	22	4.7	6
LW174	9	0	4.6	8		G85	5	22	4.6	6
Lp73a	9	4	9	8		G89	5	22	4.6	6
Lp72a	9	4	5.3	8		G64	5	22	4.5	6
Pi82	18	90	5.3	8		G82	5	23	4.8	6
Pi96	18	100	4.5	8		Lp73a	13	123	5.6	6
Lp91a	2	60	4.7	7		G89	18	57	5.6	6
Lp95a	3	59	4.5	7		G79	18	57	5	6
G76	5	22	5.5	7		G85	18	59	5.1	6
Lp72a	5	23	5.8	7		G82	18	59	5	6
Lp71a	8	22	7.4	7		Lp75a	2	87	5	5
Drc82	9	0	4.8	7		Lp75a	4	14	5	5
Lp75a	9	4	6.5	7		Lp73a	5	23	5	5
Lp74a	9	10	7.2	7		Lp73a	8	15	5.5	5
Lp71a	9	10	6.5	7		Lp74a	13	123	5	5
Biom5359	11	102	4.6	7		G76	18	59	4.5	5
Drc72b	12	80	4.6	7		Lp73a	2	46	4.5	4
Pi93	16	5	5.5	7		Lp81a	5	24	4.7	4
Gai1218	16	7	4.6	7		DB-index	3	60	p<0.005	-
Lp83a	18	94	5.3	7		DB-index	8	73	p<0.005	-
Lp71a	2	59	5.2	6		DB-index	18	92	p<0.005	-

Table 65 provide a brief characterization of the 48 QTLs, with the range in the position, the number of cases of detection, and the traits concerned by the QTLs.

Table 65 : Characterization of the 48 QTLs. Denominations, extreme positions, numbers of cases of detections, and names of the traits.

QTL	Low	High	Nb	Traits
g1-5	-	-	1	Rsh92 (thiol content; latex diagnostic)
g1-45	-	-	1	LW296 (plugging index, W2 trait)
g1-80	66	85	7	Seven Rsh traits (among 9)
g2-60	45	61	7	Seven production traits (among 14)
g2-87	-	-	1	Lp75a (production)
g2-126	-	-	1	Pi93 (inorganic phosphorus; latex diagnostic)
g3-18	-	-	2	Bark thickness (B5p, B6p)
g3-34	-	-	1	Def66c (defoliation earliness)
g3-60	49	69	47	Girth, W1, PI, Leaves, Die-back, Bark, Mn, Mz, Mz+1, R21
g3-108	-	-	1	Def31 (defoliation earliness)
g4-13	13	15	5	LW273, LW274, PI74 (plugging index), Productions Lp75a, Lp91a
g4-77	-	-	1	Def79c (defoliation earliness)
g5-16	-	-	1	Hi1723 (very dry season D2)
g5-21	17	24	13	Production, Growth during tapping, Bark thickness, Defoliation
g7-20	-	-	3	Leaves dimension
g7-66	-	-	1	Pi93 (inorganic phosphorus; latex diagnostic)
g7-105	-	-	4	Lp95a, Lp96a, Pi96, Growth before tapping
g8-21	15	22	6	Lp71a, Lp73a, Pi92, Pi95, LW173, LW174
g8-58	-	-	1	Pi82 (inorganic phosphorus; latex diagnostic)
g8-73	-	-	1	Die-back index
g8-81	-	-	1	R21 (percentage of short rubber chains)
g8-84	-	-	1	Growth before tapping (H30)
g9-4	0	10	11	Eight production traits, LW173, LW174, Drc82
g9-26	-	-	1	Growth during tapping (H79)
g9-53	-	-	1	LW296 (plugging index, W2 trait)
g10-68	66	71	6	Six defoliation traits
g10-85	-	-	1	R21 (percentage of short rubber chains)
g10-100	-	-	1	Growth before tapping (Hi2330)
g10-120	-	-	1	Def43 (defoliation earliness)
g11-35	-	-	1	Rsh93
g11-102	-	-	1	Biom5359 (biomass)
g12-20	-	-	1	Growth before tapping (Gai1218)
g12-32	20	33	5	Growth before tapping (Gai5359, biomass), Defoliation
g12-48	-	-	1	Pi93 (inorganic phosphorus; latex diagnostic)
g12-80	-	-	1	Drc72b (dry rubber content; latex diagnostic)
g12-90	-	-	1	Rsh72b (thiol content; latex diagnostic)
g13-123	-	-	2	Lp73a, Lp74a (production traits)
g14-50	-	-	1	R21 (percentage of short rubber chains)
g15-53	-	-	1	R21 (percentage of short rubber chains)
g16-6	0	9	60	Production, Drc, Pi, Rsuc, W1, W2, PI, Growth-tap, Bark, Mn, Ip, Gel
g16-42	-	-	1	Bm (Bark thickness)
g16-46	-	-	1	RSuc72b (sucrose content; latex diagnostic)
g16-60	60	61	2	B4a, Bma (Bark thickness)
g17-30	29	30	3	Three defoliation traits
g17-46	-	-	1	Pi95 (inorganic phosphorus; latex diagnostic)
g17-63	63	83	2	Drc96 (dry rubber content; latex diagnostic)
g17-84	-	-	1	Hi3643 (height increment in a period of stress, season R4)
g18-59	57	60	6	H1a, Growth during tapping
g18-94	90	100	6	Pi82, Pi93, Pi95, Pi96, Lp83a, Die-back index

These 48 QTLs provide a first mapping of the genetic hot-spots related with traits of interest in the Wickham population, and they will be a reference for comparison with future QTL-mapping studies (table 66).

Table 66 : Distribution of the 48 QTLs over the genetic map of the Genmap family.

Length (cM)	g1	g2	g3	g4	g5	g6	g7	g8	g9
0	g1-5								g9-4
10			g3-18	g4-13	g5-16				
20					g5-21		g7-20	g8-21	g9-26
30			g3-34						
40	g1-45								
50								g8-58	g9-53
60		g2-60	g3-60				g7-66		
70				g4-77				g8-73	
80	g1-80	g2-87						g8-81	
90								g8-84	
100			g3-108				g7-105		
110									
120		g2-126							

Length (cM)	g10	g11	g12	g13	g14	g15	g16	g17	g18
0							g16-6		
10									
20			g12-20						
30		g11-35	g12-32				g16-42	g17-30	
40			g12-48				g16-46	g17-46	
50					g14-50	g15-53			g18-59
60	g10-68						g16-60	g17-63	
70									
80	g10-85		g12-80					g17-84	
90			g12-90						g18-94
100	g10-100	g11-102							
110									
120	g10-120			g13-123					

We provide hereafter a synthesis of the detected QTLs by type of trait.

Growth

A total of 13 QTLs were detected for growth (girth, biomass, height). The major QTL g3-60 was associated with the growth in girth, with maximum effect during the most favorable growth periods. It was also associated with growth during tapping, bark thickness, leaves dimensions, and the die-back index, as well as with

plugging index and the W1 trait. Moreover, it was associated with traits of the macromolecular structure of rubber (M_n, M_z, M_{z+1}, and R21).

Among the other QTLs associated with growth, the QTL g5-16 was specifically associated with the height increment in the very dry season D2 (Hi1723). The QTL g5-21 was associated with six of the seven successive girths during tapping. The QTL g18-59 was also repeatedly associated with the growth in girth during tapping. The QTL g17-84 was specifically associated with the height increment in the period of stress R4 (Hi3643). The other QTLs associated with growth were g7-105, g8-84, g9-26, g10-100, g11-102, g12-20, g12-32, and g16-6.

Production

The major QTL g16-6 was associated with latex production (Lp) and many other related traits (Inorganic phosphorus, Drc, Sucrose, W2, W1, Plugging index, PI, Growth during tapping). It was also detected for traits of the macromolecular structure of rubber (M_n, I_p, Gel), and for traits of bark thickness.

Ten other QTLs were associated with girth-adjusted production traits : g2-60 (detected for seven production traits), g2-87, g3-60, g4-13, g5-21, g7-105, g8-21, g9-4 (detected for eight production traits), g13-123, and g18-94. The QTL g7-105 was detected for the last production traits Lp95a and Lp96a, and might be associated to the onset of physiological limitations due to intensification, or to the girth-size of the genotypes.

The other QTLs concerned by these traits were g1-45, g2-87, g2-126, g7-66, g8-58, g9-53, g12-48, g12-80, g16-46, g17-46, and g17-63.

Sucrose

Concerning sucrose content in the latex, only the QTL g16-46 was found specifically related with sucrose content (apart from g16-6). Moreover this QTL g16-46 showed a rather small effect, which was somewhat deceiving in regard with the importance of sucrose trait for early selection.

Thiol

Interestingly, the QTL g1-80 was detected repeatedly for 7 out of the 9 Rsh traits. But thiol content was not really used in selection so far because its capacity to predict a better tolerance to abiotic stress, and notably tapping and stimulation, is still uncertain. The other QTLs detected for Rsh traits were g1-5, g11-35, and g12-90.

Macromolecular structure of rubber

Concerning the traits of the macromolecular structure of rubber, the molar mass in number M_n was associated with g3-60 and g16-6. Moreover polydispersity and the percentage of gel were associated with g16-6. But the most interesting result was the association of the percentage of short chains, R21, with the five QTLs g3-60, g8-81, g10-85, g14-50, and g15-53.

Defoliation earliness

The QTL g10-68 was associated repeatedly with six traits of defoliation earliness of three different years. The other QTLs associated with these traits were g3-34, g3-108, g4-77, g5-21, g10-120, g12-32, and g17-30.

Bark thickness

The QTLs associated with bark thickness were g3-18, g3-60, g5-21, g16-6, g16-42, and g16-60.

Die-back

The only QTL associated with the die-back index was g18-94.

Leaves dimensions

The QTLs associated with the leaves dimensions were g3-60 and g7-20.

3.5. Selection among the 196 progenies

The measurements of growth, production, and other traits from this trial, similar to a Small Scale Clonal Trial, were available for selection among the 196 progenies. Although the objective of this trial was not to use the markers for selection, it was possible to use the genotypic data at the two major QTLs g3-60 and g16-6 for eliminating the genotypes belonging to the less favorable genotypic classes.

Among the many possible approaches, the method used hereafter was a multitrait selection, by « independent culling levels » (ICL). Although a selection based on an index would be theoretically more efficient, the ICL method was justified by the fact that we do not really know which combination of the criteria would be the most predictive of the long-term performances of the rubber clones. This ICL method aims at reducing the risk of selecting clones with an important drawback on one or the other of the selected traits. On the basis of physiological knowledge, we adjusted the mean sucrose content in the latex (Suc07a) to the production trait p7191a, and we applied the highest rates of selection to Suc07a, P7191a, and G59 in decreasing order. Lower selection rates were applied to the other criteria. The nine selection criteria which were chosen are presented in table 67.

Table 67 : Nine criteria for the selection of 20 genotypes.

Criterion	Name	Definition
S1	P7191a	Production from P71 to P91, adjusted on G59
S2	Suc07a	Mean sucrose content in 2007, adjusted on P7191a
S3	G59	Girth before tapping
S4	DB	Die-back index
S5	Nb	Number of living trees
S6	g3-60	Genotype of the QTL g3-60 at the marker a312
S7	g16-6	Genotype of the QTL g16-6 at the marker a131
S8	Gi5989a	Girth increment from 59 to 89 months, adjusted on G59
S9	P9296aa	Production from P92 to P96, adjusted on G59 and on P7191a

Table 68 shows that the three criteria S1, S2, and S3 were independent from each other. Gi5989a was negatively correlated with P7191a, and P9296aa was positively correlated with Suc07a and Gi5989a.

Table 68 : Correlations between G59, Gi5989, P7191a, P9296a, and Suc07.

Traits	P7191a	Suc07a	G59	Gi5989a
Suc07a	ns			
G59	ns	ns		
Gi5989a	-0.45	ns	ns	
P9296aa	ns	0.26	ns	0.31

For taking into account the 9 criteria, we proceeded by elimination of a certain number of genotypes for each criterion (table 69). Visual examination in the field should be done for completing this selection.

Table 69 : Number of genotypes independently eliminated for each of the nine selection criterion (Independent Culling Levels). Elimination can be carried out on more than one criterion.

Criterion	Number
S1	92
S2	100
S3	60
S4	45
S5	23
S6	40
S7	52
S8	20
S9	20

Table 70 : Comparison of the selected set of 20 genotypes to the population and to RRIM600.

		P7191a	Suc07a	G59	Die-back-ind	Nb trees	Gi5989a	P9296aa
	Units	cg	mM	cm	-	-	cm	cg
RRIM600	Mean	4304	19.43	30.84	0.149	8.5	28.01	173708
Population	Mean	4023	19.73	31.47	0.127	11.4	27.24	151431
	Max	8478	27.50	34.35	0.513	15.0	32.12	198235
	Min	1414	14.11	29.28	0.000	4.0	23.39	95578
Selection	Mean	5142	22.27	32.10	0.073	12.6	26.96	162104
	Max	6926	27.45	33.38	0.183	15.0	28.92	191556
	Min	3647	20.01	31.15	0.000	10.0	25.54	145983

Figure 33 : Productions and sucrose levels of the 20 selected genotypes, compared to RRIM600 and genotypes with the lowest and the highest sucrose levels.

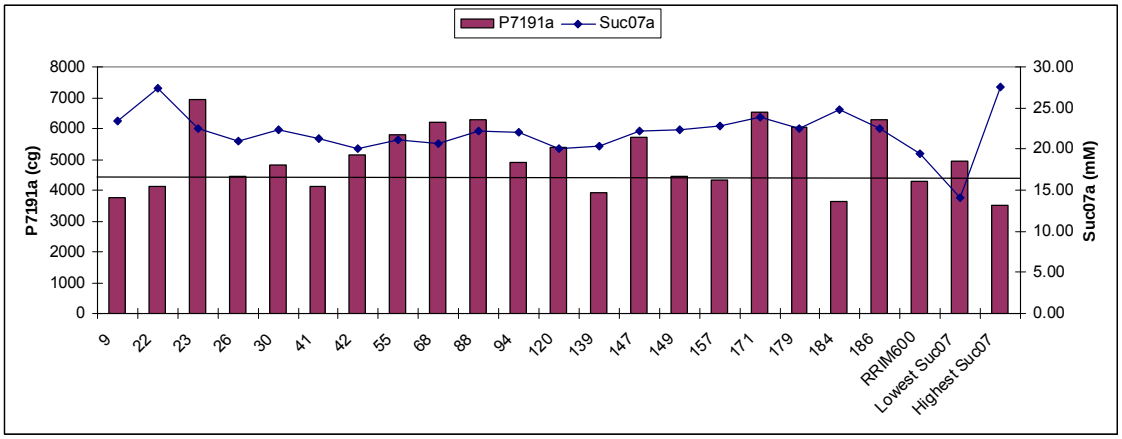


Table 71 : Values of the 20 genotypes for 7 out of the 9 selection criteria.

Codec	P7191a	Suc07a	G59	Die-back-ind	Nb trees	Gi5989a	P9296aa
9	3763	23.43	33.28	0.104	11	26.75	150520
22	4126	27.45	31.76	0.014	13	25.96	147354
23	6926	22.51	31.59	0.067	14	26.41	177836
26	4431	21.05	32.55	0.014	13	28.92	145983
30	4812	22.28	32.42	0.167	12	27.89	155284
41	4140	21.26	31.94	0.143	14	28.35	152232
42	5136	20.09	33.19	0.017	10	27.08	181605
55	5809	21.14	31.66	0.038	13	26.71	152669
68	6215	20.71	31.15	0.150	14	25.76	168936
88	6275	22.24	33.38	0.013	15	26.73	191556
94	4888	22.10	31.41	0.086	13	27.27	176757
120	5401	20.01	32.39	0.125	12	25.54	166065
139	3933	20.32	32.02	0.000	14	26.42	159850
147	5724	22.14	31.94	0.131	12	26.31	155790
149	4443	22.31	31.40	0.071	11	28.29	168451
157	4314	22.76	32.35	0.183	11	25.73	147219
171	6541	23.95	31.81	0.015	12	27.18	152656
179	6031	22.47	31.67	0.047	14	26.47	169052
184	3647	24.82	32.36	0.000	12	28.47	163096
186	6278	22.43	31.70	0.079	11	27.02	159176

For ranking all the 196 genotypes, a number of 9 points was initially given to each genotype. A genotype lost one point for every elimination on one criterion. Only the 20 selected genotypes kept the 9 points. The other genotypes ranked from 8 to 3 points.

4. DISCUSSION

A number of 48 different QTLs were detected for growth (girth and height before and during tapping), latex production and associated traits (growth during tapping ; Drc, Suc, Pi, and Rsh ; plugging index traits), traits of the macromolecular structure of native rubber (MMD_0), and secondarily for other traits such as die-back index, bark thickness, defoliation earliness, and leaves dimensions. The present discussion is focussed on the results about growth, latex production, and MMD_0, as well as on the possible use of these results for Markers-Assisted Selection.

4.1. Growth

4.1.1. Variations with successive growth periods

The experimental site was characterized by water limitations to the trees, and variations in rubber genetic response of growth to water stress were shown. In spite of various ecophysiological studies, there are very few references and results for recommending rubber clones adapted to drought prone areas. So far, ecophysiological methods were not extended to genetic studies, and there was no comparison of many genotypes in water-stressed and optimal irrigated conditions. In the present research, we examined the variations of growth fastness in relation with the alternance of rainy and dry seasons and during a drought period.

Only a moderate genetic variability was observed for growth among the genotypes, due to the full-sib nature of the family, and to its origin in the Wickham population. The mean growth characteristics of this experiment are recalled hereafter.

- At 5 years of age, the trees reached an average height of 8 meters, and a girth of 31 cm. At 7.5 years, after 15 months of early tapping, they reached a height of 11 meters and a girth of 46 cm. With the usual girth standard of opening (tapping initiation), industrial tapping would have begun at 7 years and 4 months after planting, one and a half year later that the usual age in

favourable conditions. From 18 to 59 months, the shares of cumulated girth increment were of 78 % during 21 months of rainy seasons (0.80 cm/month), and 22 % during 20 months of dry seasons (0.23 cm/month).

- Tapping was carried out at an older age during the three rainy seasons R6, R7, and R8 favourable to fast-growing. For the low-intensive tapping periods of 2007 and 2008, girth increment was of 0.90 cm/month, but it was of only 0.40 cm/month for the intensive tapping period of 2009. During the 2 untapped dry seasons D6 and D7, girth increment was of 0.22 cm/month.

- Fresh total biomasses of 130 and 255 kg per untapped tree were observed after 5 and 7 years respectively. 92 % of the total biomass produced after 59 months had been formed during the four rainy seasons R2, R3, R4, R5, and the dry season D5. In terms of biomass, growth during the first year was negligible. During this initial period, the main stake was the settlement of the living stand. The contributions of the dry seasons D2, D3, and D4 to biomass formation was very limited. A faster growth was observed in the dry season D5, due to a longer period (6 months), the relatively good rainfall of this season, and probably the development of the roots deeper than the level of the lateritic hardpan.

- Whereas the evolution of girth was marked by a regular alternance of large and small increments depending on the alternating rainy and dry seasons, growth in height was very slow all along the drought period from month 18 to 43 (seasons D2, R3, D3, R4), and the height increment during the subsequent rainy season R4 was quite nul. We assume that the terminal buds were affected by the drought.

Water stress during the rainy season R1 following planting was indicated by the short length of the second growth unit built just after planting, and the important loss of trees at the end of this season. The variations of the relationships between the increment traits of the successive seasons before tapping, and the large number of non-significant correlations for some periods, notably dry periods, suggest differential responses of the genotypes to the variations of the

environment. Whereas a positive correlation between growth in girth and growth in height was usually observed, this relationship became lower (R1) or non significant (D2, R3, D4) in periods of water stress, due to the almost cessation of growth in height. However a significant positive correlation between the two increments was observed in the very dry season D3. Another approach was to examin the correlations between the growth increments of the successive periods and the last measurements of height and girth before tapping (H53 and G59) which indicated the global growth pattern of the genotypes. Whereas the growth of the genotypes in seasons D1, R2, R3, D4, R5, and D5 were in accordance with the global trend (positive correlations), it was not the case for the seasons R1, D2, D3, and R4, thereby indicating a differential growth response of the genotypes during these periods.

Concerning growth during tapping (in rainy periods), there were in all cases positive correlations between girth increments, thus suggesting a stable genetic response to one same effect, in fact tapping and latex production. There were negative correlations between these girth increments and the production traits during the low-intensive tapping period, under the effect of diversion of assimilates from growth to latex production. In 2009, where intensive tapping generated a reduction in genetic variability of the production, girth increments were strongly reduced by intensive tapping, and there was no more correlation between these girth increments and the corresponding productions.

Heritabilities at individual tree level (h^2I) of the height traits were lower than 0.20. Heritabilities of the girth traits before tapping were lower than 0.15, but after the beginning of tapping, these heritabilities were higher than 0.20, and reached 0.27 for the last girth trait measured at the end of the research. This was explained again by the influence of latex production (a trait with a high heritability in this trial) on growth in girth.

4.1.2. Adaptation to the environment and to water stress

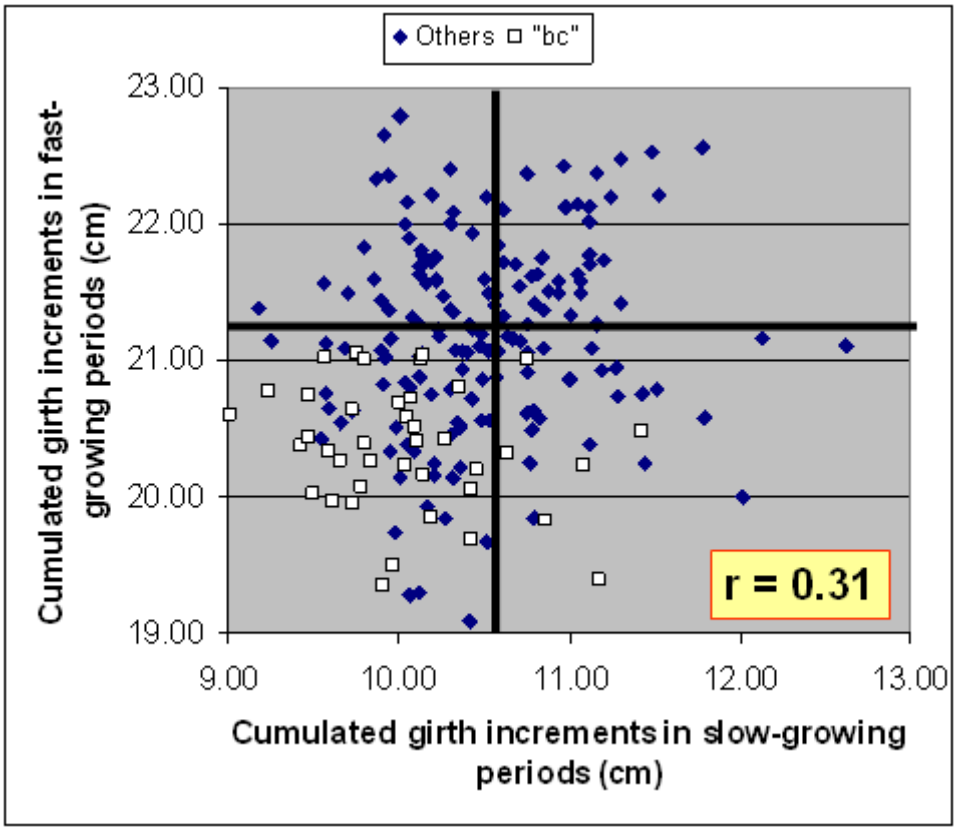
Adaptation of the genotypes to the environment of the trial may be judged partly from the ability to survive. The five genotypes n° 3, 43, 127, 181, and 195, which lost more than 50 % of their trees during the first semester after planting, were

probably more susceptible than others to water stress. With the assumption of a relationship between the die-back phenomenon and the drought, the die-back index was another indicator of genetic tolerance to water stress. But no relationship was found between this die-back index and other traits such as the number of surviving plants, or growth in height during periods of stress. On the one hand, mortality in the young age and die-back during the drought can be seen as two factors of « erosion » of the living stand. On the other hand, tolerance may be judged from the fastness of growth in girth.

The final girth before tapping G59 is an important selection criterion : fast-growing genotypes can be set to tapping earlier, thus reducing the duration of the non-productive period. For G59 to achieve a high level, was it more efficient to grow fast in the favorable or in the unfavourable periods ? For examining this point, we compared the cumulated girth increment of the four rainy seasons R2, R3, R4, and R5 (fast-growing period) with the cumulated girth increment of all the other periods with slow-growth. The fast-growing period corresponded to a duration of 27 months and to a mean girth increment of 21.04 cm. The slow-growing period corresponded to a duration of 32 months and to a mean increment of 10.35 cm. Figure 34 shows that the ranges of variation were roughly the same for the two periods : around 4 cm from the lowest to the highest genotypes. Therefore, it was theoretically possible for a genotype to make a difference on one or the other period. Although there was a slight positive correlation between the two cumulated increments ($r = 0.31$), the figure indicates that adaptations to both types of conditions contributed to the final performance of the genotypes. Therefore selection for a combined adaptation to both types of conditions would provide the best results.

With its maximum effect in the most favorable periods, the QTL g3-60 would be a selection criterion of adaptation to the fast-growing conditions. Discovering early selection criteria of adaptation to the periods of water stress would be also useful.

Figure 34 : Variations of the genotypes in the fast-growing and in the slow-growing periods. Cumulated girth increments in cm. White squares indicate the genotypes of the genotypic class « bc » for the QTL g3-60.



4.1.3. Growth and QTLs

Through the analysis of girth, height, and biomass data, before or during tapping, 13 QTLs were detected for growth, including the major QTL g3-60, and the other QTLs g5-16, g5-21, g7-105, g8-84, g9-26, g10-100, g11-102, g12-20, g12-32, g16-6, g17-84, and g18-59. Interestingly, two QTLs associated with height increments, g5-16 with Hi1723 (D2), and g17-84 with Hi3643 (R4), seemed to be expressed specifically during two periods of important stress. The effect of the QTL g3-60 on girth was important only in the favorable growth periods and it was maximum for G59, the last measured girth before tapping. This effect was due to the level of the genotypic class « bc », lower than the three other classes.

This QTL g3-60 also influenced the growth in height of the class « bc », in the same direction as for growth in girth but with a much lower effect. The QTL was significant for the height H53, only when its effect for girth was near its maximum.

By contrast, during the periods of water stress, the class « bc » was recovering from its inferiority, and might even overtake the other classes. Therefore this QTL seemed to be submitted to a genotype x environment effect. Examination of the period of the dry season D2 is particularly interesting. During this period, the increments Hi1723 and Gi1823 were low. They were not correlated between each other, and not correlated with H53 and G59 which reflect the global growth over 5 years. The QTL g3-60 was not expressed for Gi1823 but i twas almost significant for Hi1723 with an effect in repulsion (the class « bc » was higher than the three other classes). From this puzzling result, we suggest the hypothesis that another QTL, located in the neighbourhood of g3-60, exerted some specific effect on growth in height, in repulsion with the effect of g3-60 on growth in girth. The effect of this second QTL might be masked in most cases by the main effect of g3-60.

4.2. Latex production

4.2.1. Evolution of the traits, heritabilities, and correlations

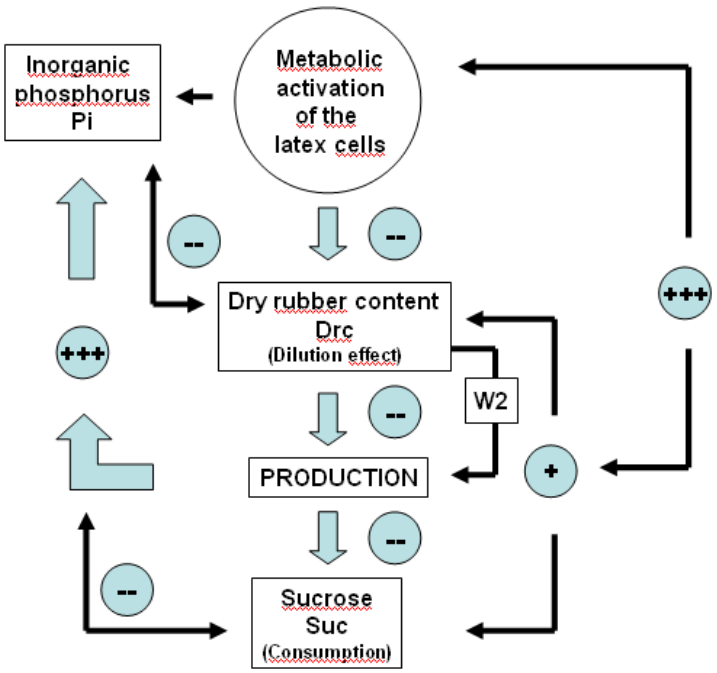
This study provided a very detailed characterization of the evolution of the latex production pattern, along a process of intensification, among the progenies of a F1 family. However there was no clear distinction of the effects of, on the one hand tapping frequency increase, and on the other hand stimulation.

For the production traits, a distinction can be done between the initial low-yielding phase from P71 to P82, and the subsequent high-yielding phase from P83 to P96. The first technical operation of intensification (the first ethephon stimulation) was applied just before P82, but with an effective effect on P83. From P82 to P83, the production was more than doubled, from 5623 mg/tree/day to 12267 mg/tree/day. Intensification was then completed by the increase in tapping frequency before P91 (from d/3 to d/2), and the application of three stimulations before P93, P94, and P96, but the mean level of production followed rather small variations from P83 to P96, just as if the genotypes were near their maximum level. However, a moderate increase in production was observed from P91 to P94, followed by a decrease in

the productions of P95 and P96 at the end of the experiment, which tends to indicate the expression of some signs of physiological limitations.

With low tapping frequency (d/3) and non-stimulated tapping, a typical pattern of latex production was observed among the genotypes, as indicated by the correlations between the traits (figure 36). Quick-starters responded to tapping by increasing the metabolism of their latex cells. As a result, Drc was decreased, thus generating a dilution effect and a longer latex flow (higher W2 parameter of the plugging index). In its turn, this long latex flow generated a more important regeneration of latex and production was increased, but at the expense of sucrose content which was decreased in the latex cells (its consumption by invertase activity was higher than its influx from the apoplast). We assume here that inorganic phosphorus, although part of a metabolic turnover, was released in higher quantity by the activation of the energetic metabolism and/or the isoprenic anabolism, and thus the balance was displaced towards a higher Pi content.

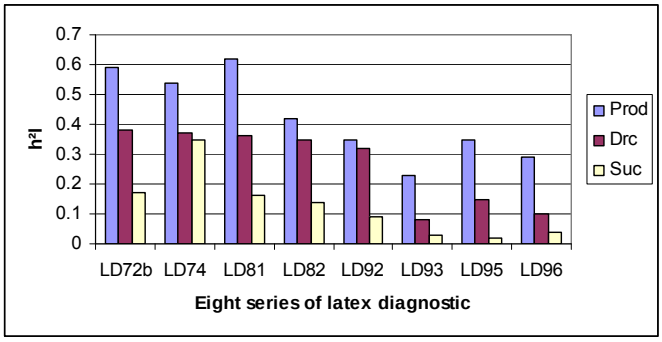
Figure 35 : Differential response of the genotypes to tapping activation of the latex cell metabolism. Relationships between latex production and Latex Diagnostic traits under low-intensive tapping.



In 2007 (low-intensive phase), latex production was strongly correlated with the W2 traits of the plugging index, confirming the positive relationship between production and the duration of latex flow. In 2009 (intensive phase), the lower level of W1 was explained by a higher tapping frequency (d/2) and a resulting lower turgor pressure in the latex cells due to a period of regeneration limited to only 2 days. The higher W2 was explained by intensification, notably stimulations, which increased the water flux into the latex cells. However the average Drc measured in the series of latex diagnostic LD92, LD93, LD95 and LD96 was not lower, which may be due to inaccurate measurements of the absolute values of these Drc traits from one LD series to the other (perhaps due to different durations of drying in owen). Whatsoever, the relative values of these Drc traits for each LD series were reliable.

With intensification, the most important effects were the increase in production and in inorganic phosphorus content, the decrease in sucrose content in the latex cells as shown in figure 35, and a general reduction in genetic variability of production traits. This reduction in genetic variability is mainly shown by the evolution of heritabilities for production, Drc, and sucrose content (figure xxx). The initial variability of Drc generated differential durations of latex flow. The genotypes with short latex flow showed a low consumption of sucrose. With intensification, Drc was decreased for all the genotypes, thus increasing the durations of latex flow and the consumption of sucrose. Although there is still some genetic variability for the production, this variability has become small for Drc and very small for sucrose content which has become a major limiting factor.

Figure 36 : Evolution of heritabilities of production, Drc, and sucrose content among eight series of latex diagnostic.



Despite of these changes, high levels of correlations were maintained between all the production traits. Intensification seemed still not « translated » into differential effects among the genotypes for production. A critical aspect concerns the possible response of the genotypes to the limitation in sucrose content. Actually, from what was observed by multiple regression, the contribution of the initial sucrose content Suc07 (2007) to the explanation of the production traits, even if not significant, followed an increasing trend and went from a negative contribution to the explanation of the level of production in the low-intensive phase, to a positive contribution in the intensive phase. This might be a sign of the ability of initial sucrose content to predict the high potential of production of some slow-starter clones, as it was shown in physiological studies (Gohet 1976).

For addressing this hypothesis which justified the initial choice of the parents of the F1 family, we examined the relationship between initial sucrose content and the evolution of the production among the genotypes. The mean sucrose content of the year 2007 was adjusted to the initial level of production in order to make it independent from the production (Suc07a). This trait was varying from 14.1 to 27.5 mM of sucrose content. Then we ordered the 196 genotypes in the increasing order of Suc07a and we tested different mobile means for examining the evolution of the mean production depending on the level of Suc07. As shown in figure 37, the group of 30 genotypes with Suc07a comprised between 21.3 mM and 22.6 mM (S30) had the highest cumulated production after intensification (cumul of the girth-adjusted productions from P93a to P96a).

Figure 37 : Evolution of the mobile mean (for sets of 30 genotypes) of the cumulated girth-adjusted production from P93 to P96 (Cum-P9396a), in relation with the increase in the initial production-adjusted sucrose content (Suc07a).

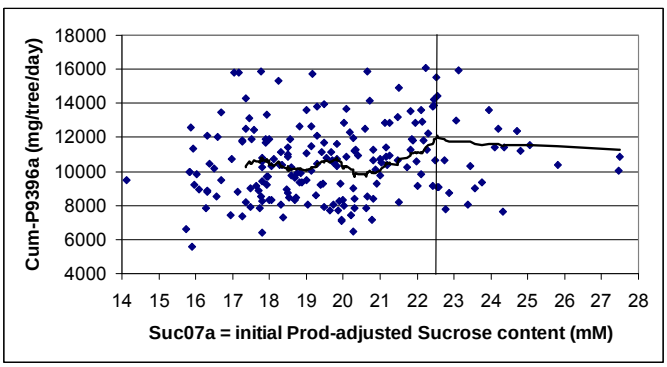
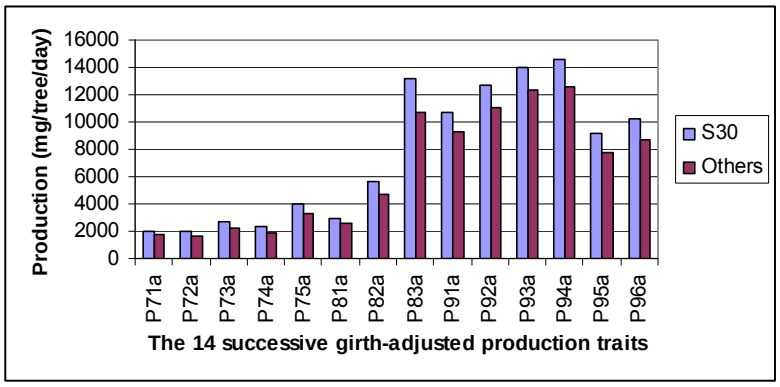


Figure 38 shows the comparison of productions between the group S30 and the 166 other genotypes. In fact, it appeared that this group had a production from 13 % to 27 % higher than that of the other group, whatever the production trait, in low-intensive as in the intensive phase. This result tends to indicate that a high level of Suc07a is favorable to a high production as soon as from the beginning of tapping. One objective of this study was to confirm that the genotypes with a high Suc07a would have a higher production under intensive conditions. The duration of the intensifive phase was probably too short for providing this confirmation.

Figure 38 : Comparison between the 30 genotypes of the group S30 and the other genotypes for their mean girth-adjusted productions.



Physiological studies of quick-starter clones like PB260 and of slow-starter clones like PB217 have shown that intensification generates new physiological phenomenons. This suggests that the situation observed in this experiment at the end of 2009 is a transitory stage between two types of genetic determinism of rubber production.

The significant negative contribution of Rsh07 in the multiple regression explaning the last productions P95 and P96 was puzzling. Therefore, in this experiment, higher the initial Rsh content in the latex and lower the production at the end of the intensive phase.

4.2.2. Production and QTLs

The discovery of the exceptional effect of the major QTL g16-6 was the most important result from this research. This QTL was shown to be mainly related with the differential metabolic activation of the latex cells, as indicated by inorganic phosphorus content, as well as Drc and sucrose content.

The reduction in the effect of g16-6 for all the traits associated with this QTL (production, Suc, Pi, Drc, plugging index) was linked with the observed reduction in genetic variability of the production and the other related traits during the intensive tapping period. And the higher dependence of production to the size of the trees explained the higher effect of the QTL g3-60 on non-adjusted production traits.

Apart from the major QTL g16-6, the two QTLs g2-60 and g9-4 were detected repeatedly for 7 and 8 girth-adjusted production traits respectively until Lp94a. By contrast the QTL g3-60 was detected for Lp95a (although this trait was adjusted to G59), and the QTL g7-105, was detected for Lp95a and Lp96a. This QTL g7-105 might be associated to the size of the trees (one QTL was already found at this locus for the height H47), or to physiological limitations due to intensification.

One « negative » result of this research concerns the absence of detection of any important QTL associated with sucrose content in the low-intensive phase. As a matter of fact, this trait is an important selection criteria for the identification of slow-starter clones with a high production potential in the long term. Apart from g16-6, only the QTL g16-46 was specific of Suc, with a moderate effect (%exp = 12 %). The male parent of the family, PB217, was specifically chosen for addressing the search of a QTL associated with sucrose content. If one important gene exists for this trait, it may be homozygous in PB217, and in this case undetectable in the progenies of PB217. Therefore, other families should be investigated.

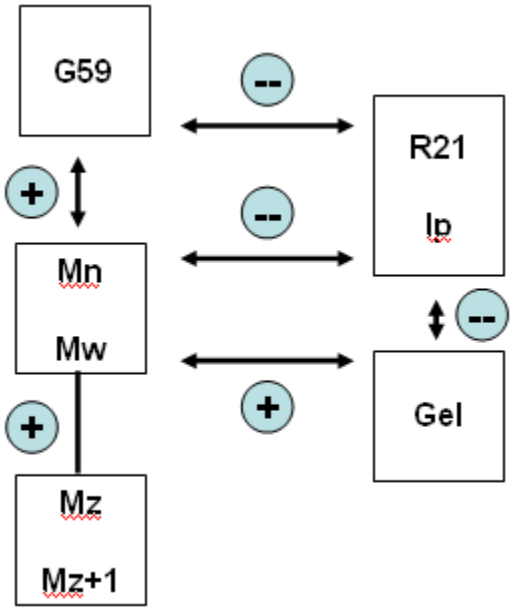
4.3. Molar mass distributions

The application of QTL-mapping to the traits of the Molar Mass Distributions (MMD_0) of native rubber was facilitated by the fact that only small quantities of latex had to be collected from young trees with low production. But phenotyping by SEC requested 5 person/months for achieving the 1057 analyses of 374 latex films.

Another difficulty was the important percentage of gel which was found after solubilisation of the latex films (59 %). Actually the elimination of this gel fraction was probably generating a bias in the results. This large gel fraction might be due to the fact that the latex films were issued from young trees (six years of age) tapped only three months before latex collection for the production of the films. The addition of hydroxilamine sulfate (HAS) was aimed at reducing this gel fraction. But for avoiding coagulation of the latex, the mixing of the latex with HAS was done very gently, and the chemical might not be homogeneously mixed with the latex, thereby limiting its effect. However, one interesting result was that this percentage of gel was found heritable ($h^2l = 0.31$) and negatively correlated with the level of production. The only QTL detected for the gel trait was the major QTL g16-6 associated with the production, and the same negative relation was found between the percentage of gel and the level of rubber production for the effect of this QTL.

Rather good levels of heritability were found for MMD_0 traits. The highest heritability was observed for R21, the percentage of short chains, thus confirming the interest of this trait for distinguishing monomodal and bimodal genotypes. Figure 39 recalls the main relationships between the traits. Latex production was positively correlated with I_p and M_z, but negatively correlated with M_n and Gel.

Figure 39 : Main relationships between the traits associated with the macromolecular structure of native rubber. Df = 194, threshold r = 0.14 for α = 0.05.



Tangpakdee et al (1996) suggested that M_n was increased with the age of the trees. Here, at the same age of the genotypes, a positive correlation was found between M_n and G59, and moreover the QTL g3-60 was detected for M_n. Therefore M_n would be positively correlated with the size of the trees, which of course is increased with age.

Polydispersity (I_p) characterizes the dispersion of the molar mass distribution, and it is normally higher for bimodal rubber. This was confirmed by the positive correlation between I_p and R21.

The most interesting result of this study was that the main trait, R21, could be explained for half of its phenotypic variance by a set of 5 QTLs, each having roughly the same effect (%exp around 10 %), including the QTL g3-60 and four QTLs specific to R21. Therefore, if this genetic determinism was confirmed by other studies, it might be possible to develop molecular selection on this trait, at least at initial stage, as a substitute to the costly SEC measurements for screening large number of genotypes.

4.4. Towards Markers-Assisted Selection in rubber

These results of QTL-mapping in only one family of the Wickham population may appear very preliminary. Although 48 QTLs were detected among all the studied traits, it was shown that MAS would be more efficient for within-family selection by focussing on a limited number of QTLs with important effects for improving the accuracy of estimation of traits with low heritability or difficult to measure at very early stage. The two QTLs g3-60 g16-6 concern two important selection criteria (growth in girth, and latex production) and they appear well-adapted for a first application of MAS to rubber selection.

The importance of the effects of these two QTLs and the repeatability of their detection suggest that they are not associated with some specific genotype x environment interactions. The QTL g3-60 was clearly associated with the growth in girth of the trunk in the most favourable growth periods. Concerning g16-6, the accordance of the results with the well-known physiological pattern of rubber production makes reasonable the assumption that this QTL can be detectable in other F1 families. As a matter of fact, it can be waited that the relationships between QTL-alleles and marker-alleles will change from one family to the other, and that the positions of the markers and of the QTLs will not be exactly the same. This makes necessary to re-estimate the QTLs in each family, but it can be done with simplified phenotyping and targetted genotyping based on a limited number of markers. We can also assume that the QTL positions are known with enough accuracy for efficient use in selection.

It would be attractive to apply initial molecular screening among a large number of genotypes prior to the testing of the selected genotypes at field level. This would be possible in the family RRIM600 x PB217, in which the QTLs were detected and characterized. In other families, a phenotyping phase is necessary, even at early stage and with limited samples of the families, mainly for the identification of the favourable biallelic combinations of the markers linked to the QTLs. Whatsoever, combining molecular and phenotypic informations, although more costly, may show

many advantages such as a better balance between selection on the QTLs and on other loci, and a more accurate estimation of genetic values.

Normally, a rubber breeding programme should include both the selection of progenies for the release of new varieties, and the selection of parents for recombination and the creation of new genetic variability. In rubber, selection of the parents was mainly based on their own value. Building mating designs for assessing the parents based on their progenies was limited by the low female fertility of most of the clones. However many families were created, but little efforts were made for comparing them with enough accuracy. In the Wickham population, due to its limited genetic variability, within-family variance was found to be larger than between-family variance, thus encouraging the breeders to focus on within-family selection. This was reinforced by the possibility of cloning the best trees at very early stage. Moreover, this simple approach was adapted to many rubber breeding programmes which benefited from limited funding, and the importance of within-family clonal selection, if not fully determined by the biology of the species, can be considered as more or less intrinsic to rubber breeding. This aspect, and the necessity of early selection, can be seen as a favourable context for the success of MAS application at the initial stage of selection. The creation of full-sib families by hand pollination will continue to generate important and unpredictable variation within each family, due to the highly heterozygous nature of rubber tree. Therefore, a new approach focussing more on selection within large-sized families rather than on the comparison of many small-sized families, might be efficient.

It was shown that the first selection stage, the Seedling Evaluation Trial (SET) was critical for rubber selection. A high heritability was found for rubber production in the specific trial used for QTL mapping. This explains why it was possible to select for production in SET : this trait is probably the only one for which there is still enough heritability for practicing massal selection at this very early stage. Actually we can assume that the heritability of production in SET is non negligible but small. A level of correlation of 0.2-0.3 was shown between genotypes studied in SET and in SSCT (Gnagne et al. 1990). Therefore, MAS combined with SET evaluation could increase the accuracy of production estimations and selection efficiency for this trait, with a global benefit taking into account the genotyping costs.

However, there are still two problems with selection in SET. On the one hand, selection for girth is normally not possible at SET level, due to the lack of correlation between seedlings and the corresponding budded clones. On the other hand, MAS based on the QTL g16-6 would reinforce the efficiency of an exclusive selection of quick-starter clones, with no additional information on the levels of sucrose content of the genotypes. This is confirmed by the data from the present research (table 72) : if only the 42 genotypes of the « best » QTL class « bc » were selected at the QTL g16-6, only one of them would belong to the group « Suc+ » with the highest sucrose content. Therefore, it seems necessary to combine MAS with a test of sucrose content at the initial selection stage, but this test, if carried out on only one seedling per genotype, would have a poor accuracy and might be inefficient.

Table 72 : Characteristics of the four genotypic classes of the QTL g16-6. The 48 genotypes with the highest sucrose content were scored « Suc+ ». Whereas 27 genotypes of this category were found in the class with the lowest production, only 1 genotype was found in the class with the highest production.

QTL g16-6	Marker a131	Prod07 (cg)	Suc07 (mM)	Nb	Nb « Suc+ »
bc	bd	48939	17.6	42	1
bd	bc	35251	19.9	51	12
ac	ad	31722	19.7	51	9
ad	ac	23062	21.3	52	27

As an alternative, it is suggested to substitute another design to the usual SET, by budding a small number of copies of each progeny (say, 3 copies) in a so-called Clonal Evaluation Trial (CET). Genotyping would be targetted to the two QTLs g3-60 and g16-6. Phenotyping of this trial would include the measurement of girth, production and sucrose content. Such a scheme would allow early selection on an index of the three traits, combining genotypic and phenotypic informations.

If really efficient, this scheme, combined with a higher selection rate, might open new opportunities such as merging the two early selection stages (SET and SSCT). In such a case, the LSCT design (the third stage) would probably require some adaptation to the evaluation of a larger number of clones (20-40 clones).

Figure 40 shows the proposal of a modified rubber selection scheme, with the substitution of SET by a « CET » trial. Paradoxically, it increases the investment in field evaluation for the first step. This is justified by the fact that a small investment was formerly devoted to SET due to its low accuracy. For taking advantage of the new opportunity of MAS, it appears logical to reinforce field evaluation at the first step for optimizing this selection. In return, a higher selection rate can be considered, with a reduction in the number of clones in SSCT. In a more speculative approach, merging the two initial stages into one CET stage, such as shown in figure 41, can be suggested.

Figure 40 : The proposed modified rubber selection scheme.

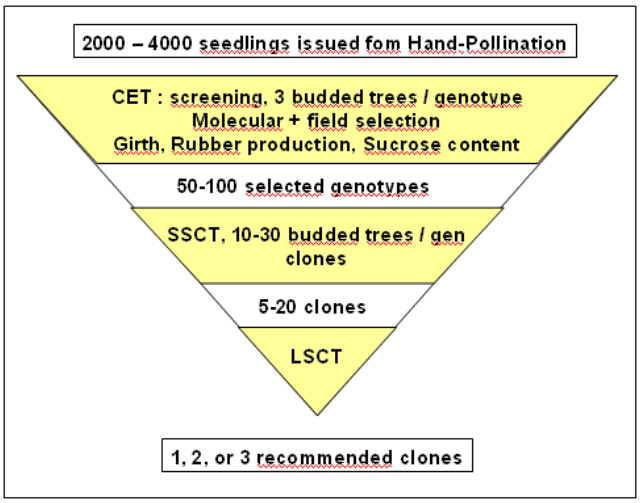
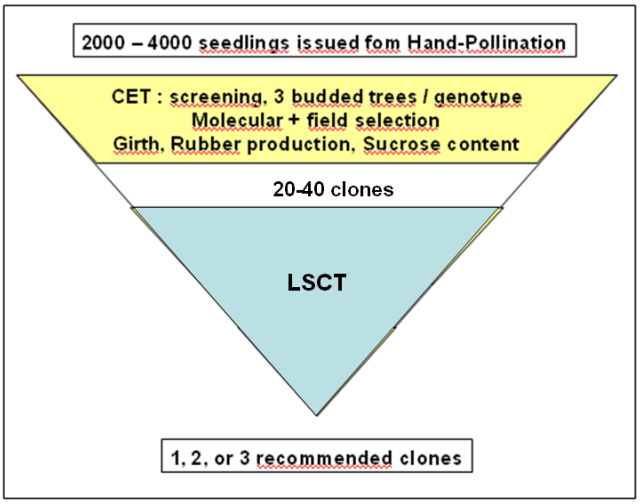


Figure 41 : A more speculative rubber selection scheme for future possible validation.



5. CONCLUSIONS

5.1. Main results

Scope of the research

For the first time in the domesticated Wickham population of the rubber tree, the characterization of a large F1 family (196 progenies) in one environment was carried out. This research included for the first time a detailed study of rubber growth in height, and a genetic study of the macromolecular structure of native rubber. The high heritability of rubber production in low-intensive conditions was remarkable, and the reduction in genetic variability with intensification provided a better understanding of the phenomenons associated with increased tapping frequencies and stimulations. QTL-mapping provided the first insight into the genetic determinism of traits of interest and of the variations of their expressions.

Growth and water limitations

The environment was characterized by a limiting annual rainfall with frequent and severe water stresses, a fast-drying soil and the presence of a lateritic hardpan. The initial planting stress was marked by a short length of the second growth-unit (first rainy season R1). Some genotypes showed a low survival rate after five months, at the onset of the first dry season (D1). At the end of a period of drought, a severe die-back, probably due to water stress, generated an important mortality and other symptoms on the trees. The analysis of growth fastness depending on the alternance of rainy (R) and dry (D) seasons was informative. Whereas growth recovery was good for girth at the onset of each rainy seasons, growth in height appeared to be more deeply and longer disturbed by drought events which probably affected the terminal buds of the trees. The variations of the correlations between growth increments suggested a diversity of genetic responses to the variations of the environment. The comparison between the cumulated girth increments of the fast-growing periods and of the slow-growing periods showed in both cases the same range of variation between extreme genotypes. Both types of conditions contributed to the final performance of growth, thus underlining the interest of a combined selection for both types of adaptation.

Rubber production

During the initial low-intensive phase of tapping, the traits related with rubber production (production itself, Drc and W2, Suc and Pi) exhibited a classical pattern of correlation among the genotypes. Quick-starters showed a higher production and a higher Pi, in relation with a lower Drc, a higher W2 and a longer latex flow after each tapping, and a lower sucrose content. Heritability of the production was very high, notably for the girth-adjusted production traits.

With intensification, the dependence of rubber production on the size of the trees (girth of the trunks) was increased, thus indicating the onset of new limiting factors such as a higher demand in assimilates. Heritabilities became lower for the production traits as well as for Drc, Suc, and Pi (latex diagnostic traits). However, the correlations between all the 14 production traits were maintained positive and high, thus maintaining the ranking of the genotypes during the intensive phase. Among the nine series of latex diagnostic (LD), for each type of trait (Drc, Suc, Pi, Rsh), all the traits of the same type were positively correlated between each other, thus showing a good repeatability of the characterization of the genotypes by the latex diagnostic. Over the whole period of production, there was a regular increase in the average inorganic phosphorus content in the latex, and a fast decrease in the average sucrose content down to a low and stable level during the intensive phase. During intensification, the heritabilities of the production traits and of Drc, Suc, and Pi, decreased a lot. This last result confirmed the recommendation of characterizing the genotypes by latex diagnostic under low-intensive tapping conditions with no stimulation, thereby in the conditions providing the highest genetic variability. The importance of the metabolic activity of the latex cells, as indicated by inorganic phosphorus content, was shown as well as the dilution effect associated with a low Drc of the latex, and the important consumption of sucrose required for generating energy (ATP) and building the rubber chains. After intensification, the stabilization of sucrose content at a low level confirmed that sucrose has probably become an important limiting factor. However, the effects of this limitation were not yet observed at the end of this study.

QTL-mapping

The studied traits included growth in girth and in height before tapping and during tapping, latex production, latex diagnostic traits, plugging index traits, susceptibility to die-back, bark thickness, defoliation earliness, leaves dimensions, and traits related with the molar mass distributions of native rubber. A total amount of 48 QTLs were detected over all the studied traits, and the main results concern the two major QTLs which were detected, g3-60 for girth, and more importantly g16-6 for rubber production. These two QTLs are currently the best candidates for the application of Markers-Assisted Selection to the screening of genotypes at very early stage. The characteristics of the QTLs provided an insight into the genetic determinism of those quantitative traits, and make a reference for comparison with further QTL mapping studies, or for mapping and search of colocalisation of candidate genes.

Another one (g7-105) might be related with a dependency of production on the size of the trees, or with the occurrence of physiological limitations to rubber production after tapping intensification.

A total of 13 QTLs were identified for growth traits, including 10 QTLs for growth before tapping (for girth or biomass : g3-60, g11-102, g12-20, g12-32, g16-6 ; for height : g5-16, g7-105, g8-84, g10-100, and g17-84), and 6 QTLs for growth during tapping (for girth : g3-60, g5-21, g16-6, and g18-59 ; for height : g8-84 and g9-26). Some of them (g5-16, g17-84) suggested a specific genetic response of growth to water stress.

The major QTL g3-60 was detected repeatedly for girth since 18 months after planting, and it explained 31 % of the phenotypic variance of the cumulated growth in girth of the trunk over the five years before tapping. Its effect on girth increment was important only during the favourable periods of growth. This effect was mainly due to the variations of one of the four genotypic classes of the QTL (class “bc”), the level of which was lower than the levels of the three other classes. This class appeared to be less adapted for growth in girth in the favourable conditions than the three other classes. The relative variations of the level of this class “bc” for growth in height seemed to “follow” the variations for growth in girth, but a special case was observed during the dry season D2, where the QTL was not detected for

the girth increment Gi1823 but close to significance level for the height increment Hi1723, with the class “bc”. It was suggested that another QTL neighbouring this locus might be specifically associated with growth in height, exhibiting a small effect in repulsion with the effect of the QTL g3-60 on growth in girth.

The QTL g3-60 was also detected for the average molar mass of native rubber (M_n) and for the percentage of short chains (R21), for the 14 rubber production traits non-adjusted to similar girth level, for the rubber production traits collected in the first five minutes after tapping (W1 in the plugging index measurement), for leaves dimensions and bark thickness, and for the die-back index issued from a scoring performed after the drought.

Eleven QTLs were identified for the girth-adjusted production traits (g2-60, g2-87, g3-60, g4-13, g5-21, g7-105, g8-21, g9-4, g13-123, g16-6, and g18-94). Three of them were detected repeatedly (g2-60, g9-4, and g16-6). The major QTL g16-6 was detected repeatedly as soon as from the first month of tapping, and it explained up to 66 % of the phenotypic variance of rubber production. It was also detected with an important effect for inorganic phosphorus content in the latex (Pi), dry rubber content (Drc), sucrose content (Suc), the traits of plugging index (W1, W2, PI), and growth during tapping. For production, there were two genotypic classes of the QTL with a medium level (“ac” and “bd”), one class with the highest level (“bc”), and one class with the lowest level (“ad”). The highest-yielding class of the QTL was also the highest for Pi and W2, but the lowest for Drc, Suc, W1, PI, and growth during tapping. Therefore the effects of the QTL totally complied with the correlations which were observed between the traits. From the low-intensive phase to the intensive phase, the effect of the QTL was considerably decreased, and even not detectable for one production trait of the intensive phase. This decrease was related with the decrease in genetic variability of the production in the intensive phase. Moreover, the non-adjusted production traits became more dependent on the girth, with an increased effect of the QTL g3-60 on the production during the intensive phase.

Apart from g16-6, only one QTL was specifically detected for only one sucrose trait (g16-46).

Four QTLs were identified for thiol content traits (g1-5, g1-80, g11-35, and g12-90). The QTL g1-80 was detected repeatedly for seven among the nine Rsh traits, explaining from 10 to 22 % of the phenotypic variance of thiol content in the latex. This trait is assumed to provide indications about the tolerance of latex cells to oxidative stress due to latex cell metabolism and/or other environmental factors. However, knowledge about is signification is still not clear enough for using it in selection.

Concerning the traits related with the macromolecular structure of native rubber, the two QTLs g3-60 and g16-6 were detected. The QTL g3-60 partly explained the three average molar masses M_n, M_z, and M_{z+1}, and it was almost significant for M_w. Moreover, g3-60 contributed to the explanation of the percentage of short rubber chains (R21). For this QTL, the genotypic class with the lowest girth also exhibited the lowest average molar masses, and the highest R21. The QTL g16-6 partly explained M_n, the percentage of gel (Gel), and the polydispersity index (I_p = M_w/M_n). For this QTL, the genotypic class with the highest yield exhibited the lowest M_n, the lowest percentage of gel, and the highest polydispersity index. The percentage of short chains (R21), considered as the best indicator of the monomodal or bimodal distributions of the molar masses of rubber, was partly explained by the five QTLs g3-60, g8-81, g10-85, g14-50, and g15-53 . Each of these five QTLs explained from 9 to 13 % of the phenotypic variance of the trait. Overall, the cumulated part of the variance of R21 explained by the additive effects of the five QTLs was of 55 %.

Eight QTLs were identified for defoliation earliness traits (g3-34, g3-108, g4-77, g5-21, g10-68, g10-120, g12-32, and g17-30). The QTL g10-68 was detected repeatedly for six of these traits, over three different years.

Six QTLs were identified for bark thickness traits (g3-18, g3-60, g5-21, g16-6, g16-42, and g16-60).

Three QTLs were identified for the die-back index (g3-60, g8-73, and g18-94).

Two QTLs were identified for leaves dimensions (g3-60 and g7-20).

From this experiment, a set of 20 new clones was selected among the 196 genotypes for final evaluation in large scale clonal trials.

5.2. Achievement of the objectives

Following the use of molecular genetic markers for genetic diversity analysis, clonal identification, and parentage testing in rubber, this research showed the potential of QTL-mapping for investigating about the genetic determinism of the main traits of interest in rubber cropping.

The discovery of the two major QTLs g3-60 and g16-6 suggest that two major genes act as limiting factors in the genetic determinism of the growth in girth during favourable conditions, and of rubber production in low-intensive tapping systems. These loci should become privileged targets for the identifcation of key-genes in rubber. The possible use of their neighbouring markers for developing Markers-Assisted Selection (MAS) in the short run appears very reasonable. It should contribute to improve the accuracy of the first selection stage which is the weakest point of the rubber clonal selection scheme.

The phenotypic study of 196 progenies of the experimental family provided the possibility to select new clones for further assessment in a Large Scale Clonal Trial. Thus QTL mapping based on a field trial corresponding to the second selection stage (Small Scale Clonal Trial) contributed to both the objectives of producing new knowledge and selecting new rubber clones.

5.3. Limitations of the research

The initial choice of the two parents was targetted towards the study of the genetic determinism of the metabolic typology of the clones, and notably that of sucrose content in the latex. In this research however, apart from the direct and causal effect of the level of production, through the QTL g16-6, only one QTL with moderate effect was detected specifically for sucrose content (g16-46). If any important QTL associated with a high level of sucrose content exists, it was probably homozygous in the PB217 parent, thus explaining that it could not be detected. Studying a F2 family issued from the recombination of some of these F1 progenies, or developing another experiment with other parents, might allow one to address this objective again in the future.

5.4. Outlook

A Large Scale Clonal Trial should be set for studying the best 10-20 clones which were selected in the experiment.

Prolongation of this research in the Genmap trial would be very useful, mainly for studying the further evolution of production in relation with the initial levels of sucrose content. Another interest would be to study the evolution of TPD rates, the observation of latex production during a dry season, or the study of vulnerability to embolism in relation with tolerance to water stress. A second series of SEC analysis of MMD_0 traits on latex films would reinforce the validity of the preliminary results.

The size of the Genmap family was of 600 progenies. Only 196 of them have been evaluated. The other part of the family may be used as an independent set of genotypes for re-estimating the effects of the most important QTLs in this family. To this end, the progenies would be genotyped at the marker-loci of the two most important QTLs, and phenotyped in a CET trial as suggested in our discussion. This operation might be extended to a set of seeds issued from natural pollination

in the center of the Genmap trial (F2 families). Other families migh be included into the CET for re-estimation of the two major QTLs followed by MAS. Application.

For the two QTLs g3-60 and g16-6, analysis of the allelic diversity at the neighbouring marker-loci should be carried out in the Wickham population. Developing research on linkage desequilibrium at these two QTLs should be considered.

Search of new QTLs in accurate SSCT phenotyping trials or in CET should be implemented for other families. The same progenies of the Genmap family, already mapped, could be studied for QTL-mapping in another environment. However, although a new genetic mapping would be necessary,the study of another family issued from two other parents in another ecological site. This might be implemented in the framework of a future project devoted to the study of the genetic variability of rubber response to water stress in Thailand.

This research should pave the way to the application of Markers-Assisted Selection in rubber. Thereby, it will contribute to the achievement of the economical and social objectives of rubber cropping, in the framework of a global context including food security and the optimization of land use.

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